Lindenberg *et al*. (2019) [ADDIN EN.CITE <EndNote><Cite><Author>Lindenberg</Author><Year>2019</Year><RecNum>14779</RecNum><DisplayText>[57]</DisplayText><record><rec-number>14779</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035601">14779</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lindenberg, F.</author><author>Sichel, F.</author><author>Lechevrel, M.</author><author>Respaud, R.</author><author>Saint-Lorant, G.</author></authors></contributors><titles><title>Evaluation of Lung Cell Toxicity of Surfactants for Inhalation Route</title><secondary-title>Journal of Toxicology and risk assessment</secondary-title></titles><periodical><full-title>Journal of Toxicology and risk assessment</full-title></periodical><pages>https://doi.org/10.23937/2572-4061.1510022</pages><volume>5</volume><number>1</number><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] evaluated the cytotoxic activity of the three nonionic polymeric surfactants Polysorbate 20 (CASRN 9005-64-5), Polysorbate 80 (Tween 80)and Poloxamer 188 (CASRN 691397-13-4), which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, in a BEAS-2B human bronchial epithelial cell model using an innovative air-liquid interface (ALI) method of exposure with a nasal spray system (MMAD and GSD not provided). In this study, the ALI results were compared to the classical submerged cell culture or liquid/liquid (L/L) model. The study measured the release of lactate dehydrogenase (LDH), an intercellular enzyme present in the cytoplasm, indicative of the loss of membrane integrity. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method;

however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to a lesser extent, Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any *in vitro* cytotoxicity.

The available *in vitro* and *in vivo* data indicate inconsistency in respiratory toxicity among nonionic surfactants; however, the degree to which the variation is due to experimental design or bioactivity of the surfactant is not discernible from these data. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties, such as surface tension or CMC. Similarly, the examination of the relationship between chemical properties of nonionic surfactants and eye irritation has not established that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths can be used to predict eye irritation potential across the nonionic surfactant subcategory [ADDIN EN.CITE <EndNote><Cite><Author>Heinze</Author><Year>1999</Year><RecNum>14780</RecNum><DisplayText>[58]</DisplayText><record><rec-number>14780</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035990">14780</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Heinze, J.E.</author><author>Casterton, P.L.</author><author>Atrash, J.</author></authors></contributors><titles><title>Relative Eye Irritation Potential of Nonionic Surfactants: Correlation to Dynamic Surface Tension</title><secondary-title>Journal of toxicology: cutaneous and ocular toxicology</secondary-title></titles><periodical><full-title>Journal of toxicology: cutaneous and ocular toxicology</full-

title></periodical><pages>359-374,
https://doi.org/10.3109/15569529909065552</pages><volume>18</volume><dates><year>199
9</year></dates><urls></urls></record></Cite></EndNote>]. However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when surface tension was measured under dynamic conditions (0.24, 1, and 4 bubbles/second). Whether this chemical property similarly predicts potency of nonionic surfactants for respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

In vivo studies

Two acute inhalation toxicity studies were identified for anionic surfactants, both demonstrated high toxicity *via* the inhalation route. Oleoyl sarcosine (CASRN 110-25-8), irritating to the skin and damaging to the eye [ADDIN EN.CITE
<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14781</RecNum
><DisplayText>[59]</DisplayText><record><rec-number>14781</rec-number><foreign-
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Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-
(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Skin
irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-

title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/4/2/?documentUUID=fbaef057-ecc7-4763-aa56-1fa2c88c606c</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], was evaluated in a 4-hour nose-only inhalation study in male and female Sprague-Dawley rats at concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L (300, 600, 2,200, 3,700 mg/m^3). The MMAD and GSD were not reported. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For sodium lauroyl sarcosinate (CASRN 137-16-6), irritating to the skin and corrosive to the eye (undiluted) [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum><DisplayText>[60]</DisplayText><record><rec-number>14782</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036284">14782</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L (50, 500, 1,000, and 5,000 mg/m^3) with a MMAD of 4.4, 2.9, 3.7, and 6.0 µm; and GSD of 2.7, 3, 4.2, and 2.9, respectively. Additionally, 5 female rats were exposed

to 1.1 or 5.5 mg/L with a MMAD 3.7 or 6.0 µm and GSD of 4.2 or 2.9, respectively [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum><DisplayText>[60, 61]</DisplayText><record><rec-number>14782</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036284">14782</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14783</RecNum><record><rec-number>14783</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036540">14783</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-

dossier/14123/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. The 5 mg/L dose resulted in fatality in all 10 animals (males and females) tested within 1-2 h of dosing and the 0.5 mg/L dose resulted in fatality for 4/5 of the males and exposure to 1 mg/L resulted in fatalities for the 10 animals (males and females) within 1-2 days of exposure. Males exposed to 0.05 mg/L did not demonstrate any adverse clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in males and females receiving concentrations of $\geq$ 0.5 mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Repeated-dose inhalation studies were identified for oleoyl sarcosine, and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (6 hours/day, 5 days/week; Organization for Economic Cooperation and Development [OECD] Test Guideline [TG] 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L (0, 6, 20, or 60 mg/m^3). The particle exposure MMAD was 1.11, 1.15, or 1.22 µm, GSD 1.68-2.57, and density 0.79 g/cm^2 for 6 hours/day, 5 days/week in 10% ethanol [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum><DisplayText>[62]</DisplayText><record><rec-number>14784</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-

title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes were observed in male animals at the high exposure concentration. In female animals of the mid-concentration exposure group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals at the mid and high concentrations, which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the alveoli walls and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The LOAEC was 0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Dioctyl sulfosuccinate sodium salt (DOSS; CASRN 577-11-7) was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex). Rats were exposed to an aerosol of a product containing 0.0042 mg/L (4.2 mg/m^3) DOSS, for 4 hours a day, 5 days a week (as reported in a secondary source; exposure details, MMAD, and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>CIR</Author><Year>2013</Year><RecNum>14785</RecNum><DisplayText>[63]</DisplayText><record><rec-number>14785</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

timestamp="1596037107">14785</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>CIR</author></authors></contributors><titles><title>Safety Assessment of Alkyl Sulfosuccinate Salts as Used in Cosmetics, Re-Review, CIR Expert Panel Meeting, June 10-11, 2013</title><secondary-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</secondary-title></titles><periodical><full-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</full-title></periodical><pages>171, https://www.cir-safety.org/sites/default/files/Sulfosuccinates_RR.pdf</pages><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]. There were no statistically significant differences in exposed and control groups for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood as indicated by elevated erythrocytic values (not otherwise specified) at 7 weeks and depressed mean corpuscular hemoglobin concentration values at 13 weeks in male rats. In females, depressed serum glutamic pyruvic transaminase and significant effect on absolute heart weight was observed at 7 weeks, depressed serum alkaline phosphatase was observed at 13 weeks and elevated glucose at 7 and 13-weeks. At 7 weeks, the lungs of necropsied animals showed scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single exposed male rat. A LOAEC of 4.2 mg/m^3 was identified based on the blood effects in male rats.

Mechanistic studies

Mechanistic studies on the pulmonary effects of anionic surfactants have been studied in dogs, rabbits, and sheep exposed to DOSS.

Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to DOSS in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Anesthetized dogs were exposed *via* a ventilator to particle sizes of 0.5 to 15 μm with an MMAD of 3 μm (no GSD reported). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Alveolar-capillary barrier permeability studies using radiolabeled aerosol tracers have evaluated whether detergents effect the surfactant layer to increase alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary elimination of radiolabeled diethylenetriamine pentaacetic acid (DTPA; CASRN 67-43-6) a relatively small hydrophilic molecule, indicating an increased alveolar permeability after detergent exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occurs with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in rabbits exposed to multiple dilutions (0.125, 0.25, 0.5, and 2%) with a MMAD of 1.7 μm of the liquid detergent [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Studies also evaluated the elimination of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser

degree than DTPA [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Wang *et al.* (1993) [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which was attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies was hypothesized to be a result of increased alveolar surface tension, which may result in increased permeability by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, as noted, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Cationic Surfactants

In vivo studies

Three acute inhalation toxicity studies were identified for cationic surfactants; one study each for DDAC, dioctadecyldimethylammonium chloride (DODMAC; CASRN 107-64-2), and BAC. DDAC, which is corrosive to the skin and severely damaging to the eye [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14786</RecNum><DisplayText>[71]</DisplayText><record><rec-number>14786</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038295">14786</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/5864/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], was tested in rats (5/sex/dose, unspecified strain) exposed *via* inhalation to 0.05, 0.09, 0.13, 0.25, 1.36, or 4.54 mg/L (50, 90, 130, 250, 1,360, or 4,540 mg/m^3) for 2 hours with an observation period of 14 days (no additional exposure conditions reported). An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2006</Year><RecNum>14845</RecNum><DisplayText>[72]</DisplayText><record><rec-number>14845</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1597755265">14845</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Reregistration Eligibility Decision for Aliphatic Alkyl Quanternaries (DDAC)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>127, https://archive.epa.gov/pesticides/reregistration/web/pdf/ddac_red.pdf</pages><volume>EPA739-R-06-008</volume><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]. A similar quaternary amine, DODMAC, which is irritating to the skin and causes serious damage to the eyes [ADDIN EN.CITE <EndNote><Cite><Author>EURAR</Author><Year>2009</Year><RecNum>14787</RecNum><DisplayText>[73]</DisplayText><record><rec-number>14787</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038841">14787</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EURAR</author></authors></contributors><titles><title>European Union Risk Assessment Report (EURAR), CAS No: 107-64-2, EINECS No: 203-508-2, dimethyldioctadecylammonium chloride (DODMAC)</title><secondary-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau

(ECB)</secondary-title></titles><periodical><full-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</full-title></periodical><pages>123, https://echa.europa.eu/documents/10162/46f2f114-12ff-4af4-8da7-72148b6a202e</pages><volume>14</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], was tested in albino rats (10 males, strain not specified) to the test substance (1:29 distilled water) *via* inhalation at 180 mg/L (180,000 mg/m^3) for one hour and observed for 14 days (no additional exposure conditions reported). No mortalities were reported and observed treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose, and labored respiration. All animals appeared normal one day after dosing. The LC_{50} (1 h) was > 180 mg/L. BAC, which is corrosive to the skin and causes severe eye damage [ADDIN EN.CITE ADDIN EN.CITE.DATA], was tested in female Wistar rats (5/group) exposed *via* nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (MMAD and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>Swiercz</Author><Year>2008</Year><RecNum>14789</RecNum><DisplayText>[75]</DisplayText><record><rec-number>14789</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039305">14789</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Swiercz, R.</author><author>Hałatek, T.</author><author>Wasowicz, W.</author><author>Kur, B.</author><author>Grzelińska, Z.</author><author>Majcherek, W.</author></authors></contributors><auth-

address>Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Łódź, Poland. radek@imp.lodz.pl</auth-address><titles><title>Pulmonary irritation after inhalation exposure to benzalkonium chloride in rats</title><secondary-title>Int J Occup Med Environ Health</secondary-title><alt-title>International journal of occupational medicine and environmental health</alt-title></titles><periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></periodical><alt-periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></alt-periodical><pages>157-63</pages><volume>21</volume><number>2</number><edition>2008/08/22</edition><keywords><keyword>Animals</keyword><keyword>Benzalkonium Compounds/administration & dosage/*toxicity</keyword><keyword>Female</keyword><keyword>Inhalation Exposure</keyword><keyword>Lung Diseases/*chemically induced/pathology</keyword><keyword>Organ Size/drug effects</keyword><keyword>Rats</keyword><keyword>Rats, Wistar</keyword></keywords><dates><year>2008</year></dates><isbn>1232-1087 (Print)
1232-1087</isbn><accession-num>18715840</accession-num><urls></urls><electronic-resource-num>10.2478/v10001-008-0020-1</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The LC_{50} was reported to be approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as tumor necrosis factor (TNF)-a, interleukin (IL)-6. Indicators of respiratory tract damage, including increased LDH, total protein, and lung weight were also observed.

Three repeated dose inhalation studies of three different exposure durations were identified for DDAC: 14-day, 28-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed *via* whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Lim</Author><Year>2014</Year><RecNum>14790</RecNum><DisplayText>[76]</DisplayText><record><rec-number>14790</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039544">14790</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lim, C. H.</author><author>Chung, Y. H.</author></authors></contributors><auth-address>Toxicity Research Team, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of didecyldimethylammonium chloride on sprague-dawley rats after two weeks of inhalation exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>205-10</pages><volume>30</volume><number>3</number><edition>2014/10/25</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword></keywords><dates><year>2014</year><

pub-dates><date>Sep</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>25343015</accession-num><urls></urls><custom2>PMC4206748</custom2><electronic-resource-num>10.5487/tr.2014.30.3.205</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The study authors reported an MMAD of 1.86 µm and a GSD of 2.75; however, individual values for each exposure concentration were not provided. Mild effects were noted in cell differential counts and cell damage parameters in BALF, in addition to inflammatory cell infiltration, and interstitial pneumonia at the medium and high exposures. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure (4-week) study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed *via* dynamic nose-only inhalation to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 DDAC (MMAD 1.4, 1.5, and 1.9 µm, GSD 1.83, 1.86, and 1.87, density not reported) for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington,

D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] . Body weights were significantly reduced in the high exposure group (males only) on days 14, 21, and 25. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. BALF analysis indicated that, at the high concentration, neutrophils and eosinophils increased with a concomitant decrease in macrophages. Histopathological findings in the nasal cavity were graded according to severity from minimal to severe and increased mucus of the respiratory epithelium in males and females was minimal to moderate at all exposures and mild to moderate ulceration of the nasal cavity in males and females in the high concentration group only. In males, there was an increase in cell count and total protein across all exposures. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. A conservative LOAEC of 0.08 mg/m^3 was previously identified by the Agency based on increased mucus of the respiratory epithelium and increased LDH; however, due to the mild effects and low number of animals/group, the effects were not statistically significant [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]

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In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole-body exposure chambers for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2017</Year><RecNum>14736</RecNum><DisplayText>[77]</DisplayText><record><rec-number>14736</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018905">14736</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, Y. S.</author><author>Lee, S. B.</author><author>Lim, C. H.</author></authors></contributors><auth-address>Chronic Inhalation Toxicity Research Center, Chemicals Toxicity Research Bureau, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of Didecyldimethylammonium Chloride (DDAC) on Sprague-Dawley Rats after 13 Weeks of Inhalation Exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol

Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>7-14</pages><volume>33</volume><number>1</number><edition>2017/01/31</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword><keyword>Sub-chronic</keyword></keywords><dates><year>2017</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>28133508</accession-num><urls></urls><custom2>PMC5266374</custom2><electronic-resource-num>10.5487/tr.2017.33.1.007</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The MMAD of the DDAC aerosol was 0.63 μm, 0.81 μm, and 1.65 μm, and the geometric standard deviations were 1.62, 1.65, and 1.65 in the low (0.11 ± 0.06 mg/m^3), the middle (0.36 ± 0.20 mg/m^3) and the high (1.41 ± 0.71 mg/m^3) exposure groups, respectively. Body weight influenced by exposure to DDAC with the mean body weight approximately 35% lower in the high exposure (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high exposure (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and LDH were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups and in males in the high concentration group only, while inflammatory cell infiltration and interstitial pneumonia was observed in both the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations of 0.8, 4 and 20 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Mean concentration of BAC in the whole-body exposure chambers of the T1 (0.8 mg/m^3), T2 (4 mg/m^3) and T3 (20 mg/m^3) groups during the exposure period was 0.84 ± 0.09, 4.01 ± 0.12, and 19.57 ± 0.97 mg/m^3, respectively; the MMAD of the aerosols was 1.614, 1.090, and 1.215 µm, respectively, and the GSD was 2.00, 1.86, and 1.51, respectively. The MMAD and GSD were confirmed to be within the range recommended by the OECD (2018) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Among the general signs observed

during the exposure period, soiled perineal region, rales, and discharge were continuously observed during the 2-week recovery period. Rales and deep respiration were observed in the high concentration. Exposure-related effects in the upper airway included nasal discharge at the low and mid concentrations, and. ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high concentrations.

In the lower airways, degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens were observed in the mid and high concentration male groups and high concentration dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchioles were observed in both males and females. Effects indicating tissue injury included squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole. In the BALF analysis, the concentration of reactive oxygen species (ROS)/reactive nitrogen species (RNS), IL-1β, IL-6, and macrophage inflammatory protein (MIP)-2 decreased concentration-dependently at the end of the exposure period, which indicated oxidative damage, but did not show a concentration-dependent change at 4 weeks of recovery. The concentrations of TNF-α, IL-4, and transforming growth factor (TGF)-β did not show changes associated with test substance exposure. Relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors identified a LOAEC of 0.8 mg/m^3 based on effects in the nasal cavity.

Mechanistic studies

In vitro assays have demonstrated that cytotoxic effects of cationic surfactants have significantly greater toxicity to non-polarized than polarized mammalian cells [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In this study, cell viability as measured by LDH and MTT assays in non-polarized HeLa immortal cell line cells and fetal skin dendritic cells (FSDC) was more sensitive to the effects of different cationic surfactants of varying alkyl chain length and polar head groups than polarized cell lines Madin-Darby Canine Kidney (MDCK) and Caco-2. The cationic surfactant toxicity was shown to occur well below their CMC, and greater toxicity was observed with alkyl lengths of 10-12 than 14-16; however, this association was not strictly a linear relationship. In addition, the cationic surfactants with a larger polar head group (*i.e.*, benzalkonium) were 2-5 times more toxic than cationic surfactants with a more localized charge (*i.e.*, trimethylammonium).

The effects of BAC on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells has been replicated *in vitro* using a dynamic culture condition that reflects the natural microenvironment of the lung to simulate the contraction and expansion of breathing [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Normal breathing levels were simulated (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of exposure rate during the cell culture. The system assessed toxicity by culturing submerged cells with different BAC concentrations (0, 2, 5, 10, 20, and 40 μg/mL) under static and dynamic culture conditions. Following a 24-hr exposure to BAC, cellular metabolic activity, IL-8, and ROS levels were

significantly affected, compared to untreated cells, when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed a higher toxic response to BAC as indicated by increased ROS levels.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The animal inhalation toxicity data identified by the literature search and PODs from the studies are summarized in [REF _Ref46931035 \h * MERGEFORMAT]. It should be emphasized that new information (*e.g.*, study data, POD derivation approaches, mechanistic information, *etc.*) may lead to updates/additions to this table. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human equivalent inhalation exposure [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-

11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. The exposure duration adjustment and DAF approaches were described above. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in [REF _Ref46931035 \h * MERGEFORMAT] for each of the toxicity studies from which PODs could be identified. However, other approaches to dosimetry adjustment may be considered relevant (*e.g.*, use of the multiple-path particle dosimetry model [MPPD]).

For the nonionic surfactant, octylphenoxypolyethoxyethanol, the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with effects in the thoracic region; therefore, the RDDR of 0.812 was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, the extrathoracic RDDR (0.0.111) was used to calculate the HEC. In the 28-day inhalation study with DDAC, effects were observed throughout the respiratory tract, including the nasal cavity; therefore, the thoracic RDDR (0.854) was used for calculating the HEC. Similarly, for the cationic surfactant, BAC histopathological cellular changes were observed in the nasal cavity and lungs, indicating the extrathoracic RDDR (0.106) should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in [REF _Ref46931035 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. Inhalation Toxicity Points of Departure and Human Equivalent Concentrations (HEC) for Surfactants.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/Type	Study POD	Value (mg/m^3)	Reference	Density (g/cm^3) at 20 °C[1]	RDDR Model Input Parameters		RDDR[2]	HEC (mg/m^3)
							MMAD (μm)	GSD		
Nonionic	octylphenoxypolyethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body	LOAEC	5.3	[ADDIN EN.CITE <EndNote><Cite><Author>MDEQ</Author><Year>2003</Year><RecNum>14731</RecNum><DisplayText>[8]</DisplayText><record><rec-number	0.998 water vehicle	1.80	1.80	$RDDR_{ET}$ = 0.196 $RDDR_{TB}$ = 1.367 $RDDR_{PU}$ = 0.564 **$RDDR_{TH}$ = 0.812** $RDDR_{TOT}$ = 1.547	1.0 7.2 3.0 **4.4** 8.2

					>14731</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018112">14731</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors					

					><author>MDEQ</author></authors></contributors><titles><title>To: Memo to File for Triton X-100 (CAS # 9002-93-1); From: Gary Butterfield; Date: November 21,2003; Subject: Screening level for Triton					

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					periodical><pages>2</pages><dates><year>2003</year></dates><urls></urls></record></Cite></EndNote>]					
Anionic	oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD TG 412)	LOAEC	< 6	[ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum><Di	0.7893 ethanol vehicle	1.16	2.12	**$RDDR_{ET}$ = 0.111** $RDDR_{TB}$ = 2.008 $RDDR_{PU}$ = 0.447 $RDDR_{TH}$ = 0.742 $RDDR_{TOT}$ = 0.970	**< 0.6** < 12.0 < 2.7 < 4.5 < 5.8

					splayTe xt>[62] </Displ ayText ><recor d><rec-number >14784 </rec-number ><forei gn-keys>< key app="E N" db-id="sp9 w2fxejs w0zre0 azr5eve arxfds0 err5sr" timesta mp="1 596036 869">1 4784</ key></f oreign-keys>< ref-type name=" Journal					

					Article" >17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl] glycine, CASRN: NA, EC number: 701-177-3, Repeate					

					d dose toxicity : Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registr					

					ation-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]					
Cationic	DDAC	4-week, 6 hr/d, 5 d/wk; nose-only	LOAEC[3] (lung effects)	0.08	[ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year>	NR	1.60	1.85	$RDDR_{ET}$ = 0.211 $RDDR_{TB}$ = 1.674 $RDDR_{PU}$ = 0.539 **$RDDR_{TH}$ = 0.854** $RDDR_{TOT}$ = 1.607	0.02 0.13 0.04 **0.07** 0.13

					<RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></f					

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					Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016<					

					/year> </dates> <urls> </urls> </record> </Cite> </EndNote>]					
	BAC	14-day, 6 hr/d, 7 d/wk; whole body	LOAEC (nasal effects)	0.8	[ADDIN EN.CITE ADDIN EN.CITE.DATA]	0.998 water vehicle 2% dose solution	1.31	1.79	**$RDDR_{ET}$ = 0.106** $RDDR_{TB}$ = 1.988 $RDDR_{PU}$ = 0.528 $RDDR_{TH}$ = 0.815 $RDDR_{TOT}$ = 0.991	**0.08** 1.59 0.42 0.65 0.79

MMAD: Mass Median Aerodynamic Diameter of inhalation study aerosol, average values listed; GSD: Geometric Standard Deviation of the inhalation study aerosol, average values listed; RDDR: Regional Deposited Dose Ration; ET: Extrathoracic; TB: Tracheobronchial; PU: Pulmonary; TH: Thoracic = TB + PU; TOT = ET + TB + PU.

[1]Exact density of administered compounds not reported (NR); vehicle density was listed when provided.

[2]RDDR values are for male and female animals, whichever was lower, as calculated using RDDR.exe and described in the Supporting Information file at "Section 2 RDDR Modeling".

[3]conservative estimate: effects were not statistically significant.

NA: Data not available or RDDR values could not be calculated from the available information.

Benchmark Margin of Exposure Analysis

The substances shown in [REF _Ref46931035 \h * MERGEFORMAT] provide representative examples of PODs that may be applied to new chemistries that meet the Surfactant Criteria, after evaluating whether the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] are appropriate toxicological analogues for read-across to the new chemical substance. Alternatively, the notifier may propose a different representative POD and/or analogue, if supported by scientific evidence. If a determination cannot be made on whether one of these chemical substances ([REF _Ref46931035 \h * MERGEFORMAT] or other representative analogue) is an appropriate toxicological analogue, then the relevant substance from [REF _Ref46931035 \h * MERGEFORMAT] should be identified as a comparator substance[4] for use in the Tiered-Testing Strategy, discussed below. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs or consideration of the representativeness and comprehensiveness of the available database to characterize potential effects after inhalation exposure. As shown in [REF _Ref46931035 \h * MERGEFORMAT], the uncertainty factors were based on RDDRs that were used as DAFs to account for animal-to-human toxicokinetic differences.

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[4] A comparator substance is one that may possess similar properties to the new chemical substance and for which inhalation toxicity data are available. EPA may "read-across" the toxicity data from the comparator substance to the new chemical substance when no other information is available. The tiered-testing approach for this category is designed to determine whether this practice may be refined or supported by additional data. As such, the comparator substance should be used in side-by-side testing in Tiers I-III with a new chemical substance to aid with interpreting the test results of the new chemical substance.

In the case of surface-active substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (See, *e.g.*, EPA, 2020 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14794</RecNum><DisplayText>[82]</DisplayText><record><rec-number>14794</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040494">14794</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>84, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2013-0605-0051&contentType=pdf</pages><volume>EPA-HQ-OPP-2013-0605-0051</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a direct-acting adverse outcome pathway (AOP) such as the one regarding surfactant that is

under development [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], the default values for UF_H and UF_A are each reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1. In order to apply these reductions, the following criteria must be established:

1. A description of the AOP,
2. A discussion of why the AOP is unlikely to differ between humans, in the case of UF_H, or between animals in comparison to humans, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is addressed by the use of dosimetry modeling.

When the above criteria are met, application of the appropriate DAF (*e.g.*, the RDDR for particles) should still be applied, given that deposition is the most appropriate dose metric for

assessing acute/subacute effects from surface-active agents. However, since the DAF accounts for the toxicokinetic component of UF_A, the remaining value of 3 (*i.e.*, $10^{0.5}$ or 3.16) should be retained for the toxicodynamics component of the UF_A.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all three of the above criteria, then a value of 3 may be applied. The reduced value represents a reduction in the TK component of this UF to 1, with the remaining value of 3 accounting for the TD component.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of dosimetric adjustments for quantifying an HEC or when the available information does not support each of the above three criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied, which represents a reduction in the TK component to 1 and application of a value of 3 for the TD component.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL with an appropriate biologically significant benchmark response (*e.g.*, 10% extra risk for quantal data or

1 standard deviation for continuous data) should be calculated and a value of 1 should be applied for this area of uncertainty.

The above considerations and approaches support the application of a benchmark MOE ranging from 10 (*i.e.*, $10^{0.5} \times 10^{0.5} \approx 10$) to 1,000 depending on the chemical substance identified as an appropriate toxicological analogue and available data on the new chemical substance. In those instances where the data are too limited to determine when one of the chemical substances is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined includes a number of uncertainties and limitations, including those associated with extrapolating the hazards identified from the chemical substances shown in [REF _Ref46931035 \h * MERGEFORMAT]. Uncertainties associated with using animals to estimate human toxicity are recognized and methods are presented to reduce extrapolation uncertainties [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2014</Year><RecNum>14795</RecNum><DisplayText>[84]</DisplayText><record><rec-number>14795</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040729">14795</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title

>Guidance on Grouping of Chemicals, Second Edition, Series on Testing & Assessment</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>141, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en</pages><volume>ENV/JN/MONO(2014)4</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. Procedures for the adjustment of exposure durations for inhalation exposures and application of DAFs to derive HECs are well-established procedures for reducing uncertainties associated with the TK aspects of animal-to-human extrapolation factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 20]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S.

Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] to new chemical substances needs to be carefully

considered, with attention given to the influence of additional functional groups on the toxicity of the new chemical substance, as well as the particle properties (MMAD, GSD, and density) of the candidate new chemical substance. Simulation studies using dosimetry models such as the RDDR or multiple-path particle dosimetry (MPPD) models can inform these considerations. Additionally, the risk assessors should consider if a different comparator substance and/or POD may be more appropriate (*e.g.*, based on new scientific information of the new chemical substance profile). Risk assessors should consider the surface tension and CMC criteria ([REF _Ref47613375 \h * MERGEFORMAT]) compared to these measurements for the new chemical substance and the influence of the presence or absence of additional functional groups on these criteria (*e.g.*, would a particular functional group increase or decrease toxicity, for example by another mechanism of action). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, the hazard(s) and risk(s) should be characterized using the chemical substance as a toxicological analogue to the new chemical substance. Of course, uncertainties regarding this extrapolation should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that a chemical substances ([REF _Ref46931035 \h * MERGEFORMAT] or other relevant analogue) is comparable to or represents an acceptable toxicological analogue to the new chemical substance, then the Tiered-Testing Strategy provided could be used to determine whether the new chemical substance has lower, comparable, or higher toxicity to the relevant chemical substance in [REF _Ref46931035 \h * MERGEFORMAT], as a comparator substance and not as a toxicological analogue. Prior to conducting such testing, the scientific

basis for selecting the comparator substance to the new chemical substance should be understood and a rationale provided as to why the comparator substance will be used for testing.

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]. Moreover, the amended TSCA requires entities undertaking voluntary testing for submission to EPA to first "…attempt to develop the information by means of an alternative test method or strategy …before conducting new vertebrate testing…" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum>

<DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]. Additionally, in 2019, EPA was directed to prioritize efforts to use NAMs to reduce animal testing [ADDIN EN.CITE <EndNote><Cite><Author>Wheeler</Author><Year>2019</Year><RecNum>14797</RecNum><DisplayText>[86]</DisplayText><record><rec-number>14797</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041176">14797</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wheeler, A.R.</author></authors></contributors><titles><title>Directive to Prioritize Effects to Reduce Animal Testing</title><secondary-title>United States Environmental Protection Agency</secondary-title></titles><periodical><full-title>United States Environmental Protection Agency</full-title></periodical><pages>3, https://www.epa.gov/sites/production/files/2019-09/documents/image2019-09-09-231249.pdf</pages><dates><year>2019</year></dates><urls></urls></record></Cite></EndN

ote>]. Multiple NAMs exist which can be used to assess hazards and risks of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing and *in vitro* methods to specifically assess respiratory toxicity. Several methods are described within a tiered-testing strategy recognizing that these assays are provided as examples and the development of NAMs is advancing rapidly. As such, the NAMs included here should not be considered all-inclusive or a final compilation. EPA strongly encourages the development and use of NAMs, particularly to reduce or replace the use of animals and is open to considering and discussing additional NAMs with PMN submitters during a pre-notice consultation [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14829</RecNum><DisplayText>[87]</DisplayText><record><rec-number>14829</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596098792">14829</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Schedule a Pre-Submission Meeting, Reviewing New Chemicals under the Toxic Substances Control Act (TSCA)</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/forms/program-contacts-and</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

In the interest of reducing or replacing vertebrate testing and designing a scientifically robust testing approach, when a surfactant is determined to be respirable, EPA encourages evaluating its potential to cause respiratory tract toxicity using an AOP approach. The OECD provides "An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect" and that "AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning" [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14798</RecNum><DisplayText>[88]</DisplayText><record><rec-number>14798</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041285">14798</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Adverse Outcome Pathways, Molecular Screening and Toxicogenomics</title><secondary-title>Organization for Economic Cooperation and Development (OECD)</secondary-title></titles><periodical><full-title>Organization for Economic Cooperation and Development (OECD)</full-title></periodical><pages>http://www.oecd.org/env/ehs/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. AOPs in various stages of development are useful for different purposes and an AOP may be useful even if it has not been formally evaluated by the OECD.

An AOP can be used to help design a testing strategy and to identify NAMs that can query the key events leading up to the adverse outcome. As an example, using the respiratory contact irritant chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CASRN 1897-45-6), Syngenta Crop Protection applied a NAM for the assessment of inhalation toxicology based on an AOP approach [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The approach involved derivation of the POD from an *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Similar approaches can be used for surfactants where *in vitro/ex vivo* systems may be used to investigate specific key events in an AOP and confirm that a new chemical substance fits within the boundaries of the Surfactant Category, and therefore, may act like a surfactant (group assignment *via* similar AOP) and/or if other substance-specific properties lead to a predominant type of key event within the AOP. Further, *in vitro* tests may deliver information while avoiding *in vivo* testing or, if considered, provide helpful information on dose-selection for *in vivo* testing.

An AOP connects a molecular initiating event (MIE) to key events, at the cellular, tissue, and organ levels, which lead to an adverse outcome at the organism or population level [ADDIN EN.CITE ADDIN EN.CITE.DATA]. For surfactants, proposed MIEs include interaction of the substance with the epithelial lining fluid or lung-surfactant, or the molecular interaction of the substance itself with cell membranes of the epithelium in the respiratory tract. The resulting key events include disruption of airway epithelial cells (AEC) due to loss of lung cell surfactant function and/or the loss of membrane integrity (cellular level key events). These cellular events may lead to different tissue or organ level events (*e.g.*, cytotoxicity and perturbation of AEC, increased alveolar surface tension and alveolar collapse, loss of barrier function, blood

extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (*i.e.*, the adverse outcome) (*e.g.*, pneumonia, limited lung function by chronic obstruction (COPD), interstitial fibrosis, *etc.*).

Some *in vitro* tests, such as by capillary surfactometer, may be useful in screening chemicals to be tested for the Surfactant Category, but do not by themselves constitute adequate tests for acute respiratory tract effects of these chemicals. This information should be taken into consideration within an integrated approach. These assays can be used as part of a weight of evidence evaluation to determine whether to consider animal testing or if a POD can be determined for risk assessment purposes without the use of animals. Each test can provide insight on one key event of the AOP, which collectively, may provide a comprehensive picture of the likelihood of toxicity.

A number of different types of *in vitro* test methods, summarized in [REF _Ref46931271 \h * MERGEFORMAT], may be used to query key events in AOPs relevant to the disruption of lung function by surfactants [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-

title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Clippinger *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA] have also described a decision tree and potential key events that can be used to design pathway-based approaches for *in vitro* testing of inhalation exposures.

Table [SEQ Table * ARABIC]. Potential Methods for Evaluating Chemicals in the Surfactant Category.

Level of Biological Organization	*Key Events*	*In Vitro Assay*	*Test System*
Molecular Initiating Events (MIEs)	Interaction with pulmonary surfactant	*In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant interaction, *e.g.*, as described by Sorli *et al.* (2018) [ADDIN EN.CITE ADDIN E
	Interaction with cell membrane and cell membrane components and interaction	Hemoglobin Denaturation Assay, Liposome Assay, and *In Vitro/Ex Vivo* Irritation Assays	• Hemoglobin denaturation assay, *e.g.*, as described by Hayashi *et al.* (1994) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1994</Year><RecNum>14838</RecNum><Display id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596732926">14838</key></foreign-keys><ref- H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Kato, S.</author></autho address><titles><title>Multivariate factorial analysis of data obtained in seven in vitro test systems for pred published in association with BIBRA</alt-title></titles><periodical><full-title>Toxicology in vitro : an inte periodical><full-title>Toxicology in vitro : an international journal published in association with BIBRA</f 20</pages><volume>8</volume><number>2</number><edition>1994/04/01</edition><dates><year>199 num>20692908</accession-num><urls></urls><electronic-resource-num>10.1016/0887-2333(94)90185-6 provider><language>eng</language></record></Cite></EndNote>]
			• Liposome assay, *e.g.*, as described by Kapoor *et al.* (2009) [ADDIN EN.CITE <EndNote><Cite><Author> number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5 type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chau Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfacta science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-t science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages> Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><key Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dye Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</access provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
			• *In vitro/ex vivo* eye irritation tests for penetrance, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayT id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref- type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstru

			irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</se https://www.oecd-ilibrary.org/docserver/9789264242548-en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF0 Bovine Corneal Opacity and Permeability Test (OECD TG 437) [ADDIN EN.CITE <EndNote><Cite><Au number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5 type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Co Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelir Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846-en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04 Isolated Chicken Eye Test (OECD TG 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Autho number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596 type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated ch irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</se https://www.oecd-ilibrary.org/docserver/9789264203860-en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E71 *etc.*
Cellular Level Events	Loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• *In vitro/ex vivo* eye irritation tests for cytotoxicity, *e.g.*, Reconstructed human Cornea-like Epithelium (RhC <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayT id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref- type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstruc irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</se https://www.oecd-ilibrary.org/docserver/9789264242548-en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF0 Bovine Corneal Opacity and Permeability Test (OECD TG 437) [ADDIN EN.CITE <EndNote><Cite><Au number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5 type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Co Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelir Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846-en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04 Isolated Chicken Eye Test (OECD TG 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Autho number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596 type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated ch irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</se https://www.oecd-ilibrary.org/docserver/9789264203860-en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E71 *etc.*

			• Cell membrane integrity test (LDH-cytotoxicity assay), cell viability assays (*e.g.*, MTT, resazurin [ADDIN membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCV <EndNote><Cite><Author>ICCVAM</Author><Year>2006</Year><RecNum>14805</RecNum><Displa id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044231">14805</key></foreign-keys><ref- type><contributors><authors><author>ICCVAM</author></authors></contributors><titles><title>In vitro title>ICCVAM Test Method Evaluation Report</secondary-title></titles><periodical><full-title>ICCVAM https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf</pages><volume>NIH
Tissue or Organ Level Events	Tissue level events	Human organotypic Airway Cultures	• 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (*e.g.*, EpiAirway™, Mu [ADDIN EN.CITE ADDIN EN.CITE.DATA]
	Tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test, *e.g.,* as described by Hess *et al.* (2016) [ADDIN EN.CITE ADDIN EN.CITI

MIEs

There may be multiple AOPs that would be relevant to the Surfactant Category. The MIE for a proposed AOP under development is the interaction of a substance with lung surfactant, which may lower the surface tension and disrupt lung surfactant function [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Sorli *et al.* (2017) [ADDIN EN.CITE ADDIN EN.CITE.DATA] developed an *in vitro* lung surfactant interaction assay that specifically measures whether a substance alters the surface tension of pulmonary surfactant. The assay was initially developed for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle that may be relevant for some types of surfactants.

The proposed MIE for another AOP relevant to surfactants is direct interaction with AEC or pulmonary cell membranes, which may be followed by cytotoxicity. While the hemoglobin denaturation and liposome assays and *in vitro* eye irritation assays do not directly measure effects on membranes of AEC, these assays have been shown to be useful screening approaches for determining the ability of surfactants to interact with cellular membrane components and cell membrane penetration. For example, Hayashi *et al.* (1995) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1995</Year><RecNum>14833</RecNum><DisplayText>[105]</DisplayText><record><rec-number>14833</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539110">14833</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hayashi, T.</author><author>Itagaki, H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Sato, Y.</author><author>Suzuki, Y.</author></authors></contributors><auth-address>Shiseido Research Center, Yokohama, Japan.</auth-address><titles><title>Hemoglobin denaturation caused by surfactants</title><secondary-title>Biol Pharm Bull</secondary-title><alt-title>Biological & pharmaceutical bulletin</alt-title></titles><alt-periodical><full-title>Biological & Pharmaceutical Bulletin</full-title><abbr-1>Biol. Pharm. Bull.</abbr-1></alt-periodical><pages>540-3</pages><volume>18</volume><number>4</number><edition>1995/04/01</edition><keywords><keyword>Chromatography, High Pressure Liquid</keyword><keyword>Circular Dichroism</keyword><keyword>Hemoglobins/*chemistry</keyword><keyword>Irritants/pharmacology</keyword><keyword>Protein Denaturation/drug effects</keyword><keyword>Sodium Dodecyl

Sulfate/pharmacology</keyword><keyword>Spectrophotometry</keyword><keyword>Structur e-Activity Relationship</keyword><keyword>Surface-Active Agents/*pharmacology</keyword><keyword>Taurine/analogs & derivatives/pharmacology</keyword></keywords><dates><year>1995</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0918-6158 (Print)
0918-6158</isbn><accession-num>7655423</accession-num><urls></urls><electronic-resource-num>10.1248/bpb.18.540</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] showed that charged surfactant molecules can interfere with charged side chains of the hemoglobin protein. These interactions led to disruption of the three-dimensional (3D) structure of hemoglobin, causing a change in light absorbance that can be measured. Increasing concentrations of SDS and sodium lauroylmethyltaurate (LMT; CASRN 4337-75-1) were tested in this assay and showed concentration dependent increases in hemoglobin denaturation, which correlated with irritation effects in the Draize eye test [ADDIN EN.CITE ADDIN EN.CITE.DATA].

The liposome assay can be used to assess disruption of the lipid bilayer of the membrane from interaction with surfactant chemistries. Kapoor *et al.* (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><RecNum>14834</RecNum ><DisplayText>[96]</DisplayText><record><rec-number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300">14834</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors></contributors><auth-

address>Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalmol Vis Sci</secondary-title><alt-title>Investigative ophthalmology & visual science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></periodical><alt-periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</number><edition>2009/01/27</edition><keywords><keyword>Conjunctival Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques, Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><keyword>Humans</keyword><keyword>*Liposomes</keyword><keyword>Luminescent Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-Active Agents/*toxicity</keyword></keywords><dates><year>2009</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electronic-resource-num>10.1167/iovs.08-2980</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] measured the release of calcein dye from liposomes following exposure to various surfactants and showed a positive correlation with these findings and data from the Draize eye test. The hemoglobin denaturation

and liposomal assays were both optimized and validated against eye irritation data; therefore, these assays may provide an opportunity to evaluate the effects of surfactants on the respiratory tract. Further *in vitro* testing of known surfactants with existing data alongside new chemical substances will help benchmark the results. Nonetheless, these assays are useful for understanding the potential toxicity of a new surfactant substance to AEC or pulmonary cell membranes.

The use of *ex vivo* eye irritation studies may provide indirect measures of surfactants on cell membranes, which may be relevant to the effects observed from comparator substances in the respiratory tract. For example, Bader *et al.* (2013) [ADDIN EN.CITE <EndNote><Cite><Author>Bader</Author><Year>2014</Year><RecNum>14807</RecNum><DisplayText>[107]</DisplayText><record><rec-number>14807</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044694">14807</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bader, J.E.</author><author>Norman, K.G.</author><author>Raabe, H.</author></authors></contributors><titles><title>Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability Assay</title><secondary-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</secondary-title></titles><periodical><full-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</full-title></periodical><pages>https://iivs.org/wp-content/uploads/2018/08/iivs_poster_predicting-ocular-irritation-of-surfactants-using-the-bovine-corneal-opacity-and-permeability-assay.pdf</pages><dates><year>2014</year></dates><urls></urls></record></Cite></EndNot

e>] reported that the Bovine Corneal Opacity and Permeability (BCOP) assay was effective at demonstrating that nonionic (*i.e.*, octylphenoxypolyethoxyethanol), anionic (*i.e.*, SDS), and cationic (*i.e.*, BAC) substances cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. The permeability score was more predictive of eye irritation than the ocular opacity score for octylphenoxypolyethoxyethanol and SDS, whereas with BAC, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation of opacity and permeability measures of surfactants tested in the BCOP may be helpful with elucidating toxicity to AEC or pulmonary cell membranes.

In addition, information on the potential of a substance to cause skin irritation (*e.g.*, OECD TG 439 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14808</RecNum><DisplayText>[108]</DisplayText><record><rec-number>14808</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044884">14808</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed Human Epidermis Test Method, In vitro Skin Irritation</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>26, https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1596045726&id=id&accname=guest&checksum=2580E92A5C8

89D0DD65599260E7866D3</pages><volume>439</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]) and/or skin corrosion (*e.g.*, OECD TG 431 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14809</RecNum><DisplayText>[109]</DisplayText><record><rec-number>14809</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044976">14809</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>29, https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1596045820&id=id&accname=guest&checksum=E3EE55CBAAFAF0432EAD109F1B39ECF0</pages><volume>431</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]) *in vitro*, can provide supporting evidence of the potential for a substance to cause similar irritant or corrosive effects in respiratory tract cells. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, demonstrated by DDAC that causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 [ADDIN EN.CITE <EndNote><Cite><Author>Sigma-Aldrich</Author><Year>2020</Year><RecNum>14810</RecNum><DisplayText>[110]</DisplayText><record><rec-number>14810</rec-number><foreign-keys><key app="EN" db-

id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045132">14810</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sigma-Aldrich</author></authors></contributors><titles><title>Safety Data Sheet, Product name: Didecyldimethylammonium chloride, Version 8.1, Revision Date: 03/28/2020, Print Date: 05/29/2020</title></titles><pages>9, https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

Cellular Level Effects

In vitro/ex vivo assays can be used to assess key events on the cellular level in AOPs relevant to the Surfactant Category (see Supplemental Table 1 in Clippinger *et al.*, 2018 [ADDIN EN.CITE ADDIN EN.CITE.DATA]). For general cytotoxicity ([REF _Ref46931271 \h * MERGEFORMAT]), cell lines are available that are known to be sensitive to the effects of surfactants. Use of the BALB/c 3T3 NRU cytotoxicity test to reduce animal testing by estimating starting doses for acute oral toxicity testing has been reviewed and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and is an OECD guidance document [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The surfactants with known inhalation toxicity (*e.g.,* octylphenoxypolyethoxyethanol, oleoyl sarcosine, DDAC, or BAC) should be tested in parallel with the new chemical substance to benchmark the results, thereby providing reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

Tissue or Organ Level Effects

Based on the results of testing cellular level key events, it may be necessary to perform additional testing. Human and animal airway epithelia are composed of multiple cell types that each have specialized functions, making the use of 3D co-culture assays more physiologically relevant than 2D monoculture systems. Thus, several human organotypic airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems. Two commonly employed systems are EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively.

Organotypic airway cultures, such as EpiAirway™ and MucilAir™, [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[112]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33,

https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain homeostasis for months in culture. Because of these characteristics, these human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Dosimetry models such as the RDDR or MPPD can be used to predict the anatomical area and internal amounts delivered in various regions of the respiratory system for humans under the target inhalation exposure scenario for the given use case. Different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. MucilAir™ provides a 3D co-culture model of cells from nasal, tracheal or bronchial sites, and SmallAir™ provides a co-culture model of cells from small airways. EpiAirway™ is composed of a co-culture of normal human tracheal/bronchial epithelial cells, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts (available with and without macrophages).

Exposure of respiratory tract 3D co-culture models to aerosols at the air liquid interface (ALI) using an *in vitro* exposure system, such as those available from Vitrocell® Systems, provides an exposure more comparable to real-life scenarios for inhaled aerosols. The tradeoff has been a lower throughput compared to *in vitro* two-dimensional exposure systems; however, 3D tissue models and ALI exposure systems are now available in a 96-well format. Dilution in medium

and interaction with medium components does not occur in the ALI exposure systems as in submerged culture systems. The respiratory tract 3D co-culture models are more physiologically relevant because there is an interaction of the aerosol with a mucus or surfactant layer, as in humans.

Exposures of these organotypic cultures at the ALI can be combined with other assays for assessing cell function and viability in an AOP approach. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays (such as MTT, resazurin, or ATP assays), have all been reported for use with these cultures. Further, multiple assays can be performed on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant octylphenoxypolyethoxyethanol at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, provided dosimetry models are available for translation of the internal dose achieved under culture conditions to an equivalent inhalation exposure for the human scenario of interest. Examples of *in vitro* dosimetry models to predict particle doses for submerged cell culture include the *In vitro* Sedimentation, Diffusion and Dosimetry model (ISDD) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and the *In vitro* Sedimentation, Diffusion and Dissolution Dosimetry (ISD3) model [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Significant progress has been made toward achieving the objectives to use high-throughput *in vitro* assays and computational models to evaluate potential adverse effects of chemical exposures [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[16, 115]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite><Cite><Author>NRC</Author><Year>2017</Year><RecNum>14812</RecNum><record><rec-number>14812</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045703">14812</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Using 21st Century Science to Improve Risk-Related Evaluations, Washington, D.C., The National Academies Press</title></titles><pages>200, https://doi.org/10.17226/24635</pages><volume>ISBNs: Ebook: 978-0-309-45351-6; Paperback: 978-0-309-45348-

6</volume><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. To translate the effects to higher levels of biological organization, a battery of assays with varying complexity and physiological relevance may be needed. The 3D human organotypic airway cultures add evidence to an AOP approach and increase confidence in the physiological relevance to humans.

Precision-cut lung slices (PCLS) provide an additional method to develop key event data using *ex vivo* cultures of human or rodent lung slices. The PCLS can be used to measure multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often used in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema [ADDIN EN.CITE <EndNote><Cite><Author>Sanderson</Author><Year>2011</Year><RecNum>14814</RecNum><DisplayText>[117]</DisplayText><record><rec-number>14814</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046031">14814</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sanderson, M. J.</author></authors></contributors><auth-address>Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA 01655, USA. Michael.Sanderson@umassmed.edu</auth-address><titles><title>Exploring lung

physiology in health and disease with lung slices</title><secondary-title>Pulm Pharmacol Ther</secondary-title><alt-title>Pulmonary pharmacology & therapeutics</alt-title></titles><periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></periodical><alt-periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></alt-periodical><pages>452-65</pages><volume>24</volume><number>5</number><edition>2011/05/24</edition><keywords><keyword>Animals</keyword><keyword>Cell Physiological Phenomena</keyword><keyword>Disease Models, Animal</keyword><keyword>Humans</keyword><keyword>Lung/pathology/*physiology</keyword><keyword>Lung Diseases/*pathology</keyword><keyword>Microscopy/methods</keyword><keyword>Muscle Contraction/physiology</keyword><keyword>Organ Culture Techniques</keyword></keywords><dates><year>2011</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1094-5539 (Print)
1094-5539</isbn><accession-num>21600999</accession-num><urls></urls><custom2>PMC3168687</custom2><custom6>NIHMS296121</custom6><electronic-resource-num>10.1016/j.pupt.2011.05.001</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be evaluated. One further advantage of PCLS is that the assay can be performed on multiple species to determine inter-species variability in susceptibility.

Human PCLS, derived from, for example, rejected but otherwise healthy transplant tissue, can be used to measure cell/tissue viability, local respiratory inflammation, and physiological function. These endpoints can be measured in single and repeated exposures in a metabolically competent system within the normal architecture of the lung in a more relevant model system, replacing the need for animal testing [ADDIN EN.CITE ADDIN EN.CITE.DATA].

When human PCLS are not available, rat PCLS provide an alternate option. The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed correlation with *in vivo* LC_{50} values [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The use of rat PCLS reduce the number of animals used to conduct dose response studies, as compared to *in vivo* inhalation tests. From a rat lung (1 g), approximately 200 slices can be prepared. In general, for each test substance concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested using tissue from a single rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures *via* liquid media or, with additional adaptations, air. As such, rodent PCLS meet the goal of reducing animal testing, although dosimetry models for their translation to HEC are not yet developed. Mechanistic rodent and human PCLS studies may be conducted in parallel to understand species specific difference in toxicological effects. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations of an AOP Approach to the Surfactant Category

A number of *in vitro* assays have been discussed as to their potential utility for assessing key events in an AOP(s) relevant to characterize the Surfactant Category. Uncertainties and limitations associated with these assays are discussed for each of the above testing systems, as well as others [ADDIN EN.CITE ADDIN EN.CITE.DATA]. It is important to consider that these assays were not systematically tested using surfactants. Nonetheless, these assays can be conducted using an AOP approach to provide information on whether a new chemical meets the Surfactant Category criteria and/or to understand whether the new chemical may be more or less bioactive or toxic than the sub-category comparator chemicals. EPA will generally use the framework and analogue toxicity data identified in this investigation to assess potential risks from surfactants.

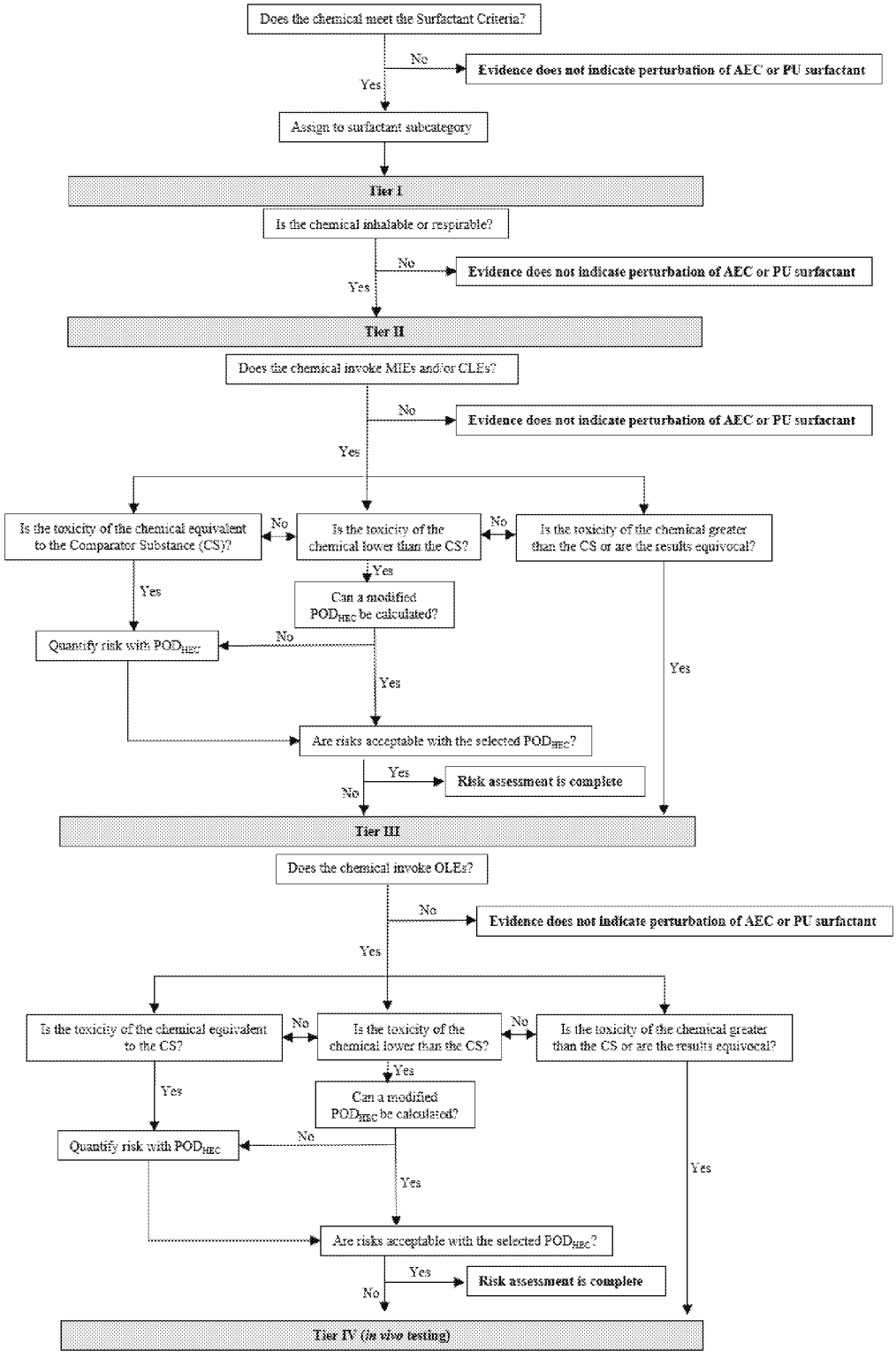
In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment continues to evolve. A fit-for-purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, and domain of applicability for evaluating a new method to inform specific decisions has emerged from the regulatory science community to address the challenges posed for validation of NAMs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Such fit-for-purpose validation approaches are intended to be flexible and adaptable and to provide data sets, prediction analysis results, inference models, *etc.* in a transparent manner that enable other scientists to confirm the performance of the assays and inference models, as well as evaluate the rationale for using these assays in a specific decision context.

Once such fit-for-purpose scientific evaluations are documented, there are several ways that these assays can be used to reduce and replace animal testing. First, testing can be performed based on an AOP approach to evaluate the potency of new surfactants versus a comparator substance within the relevant subcategory that has repeated exposure inhalation toxicity data. Second, depositional data using models such as the RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Tiered-testing Strategy

The first step in the tiered-testing strategy is to determine if the evaluated substance meets the Surfactant Criteria. If so, then assign the substance to the appropriate surfactant subcategory (nonionic, anionic, or cationic) and determine whether any of the representative subcategory chemicals may serve as an acceptable toxicological analogue for risk assessment or as a comparator substance for tiered testing. If a representative subcategory chemical is determined to be an acceptable toxicological analogue to the new chemical substance, then quantify risks using the toxicological analogue. If the MOE is equal to or greater than the benchmark MOE, then tiered testing is not required on the new chemical substance. If the MOE is lower than the benchmark MOE or if a determination cannot be made on whether any of the representative subcategory chemicals are acceptable toxicological analogues, then proceed with tiered testing using the most appropriate subcategory chemical as a comparator substance to the new chemical substance. As detailed below, the tiered-testing strategy commences with the least complex, most efficient testing methods, and at each subsequent tier, the complexity of the test system

increases, commensurate with key events in proposed AOPs relevant to the Surfactant Category, to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system. It is envisioned that both the new chemical substance and the comparator substance will be evaluated side-by-side in the NAM assays. The results of these studies may lead to the conclusion that the comparator substance is an acceptable toxicological analogue to the new chemical substance. Alternatively, the results may support that higher tiered testing is warranted, particularly when the new chemical substance has higher toxicity than the comparator substance. If *in vivo* testing is conducted, it may not be necessary to run the comparator substance in the *in vivo* tests, given that suitable inhalation studies are available on the comparator substances. A summary of the proposed tiered-testing strategy is provided in [REF _Ref48210489 \h * MERGEFORMAT] and discussed further below.



Scheme [SEQ Scheme * ARABIC]. Proposed tiered-testing strategy for general surfactants.

Tier I—Physicochemical properties

Surfactants are proposed to cause a specific sequence of biological events in the respiratory tract if they are inhaled. Manufacture, processing, or use of a surfactant in an inhalable form, (*i.e.*, ≤ 100 μm aerodynamic diameter) is therefore, an initial consideration of the potential for a surfactant to cause toxicity to the respiratory tract. Particle size is an established parameter for determining inhalability/respirability of particles/droplets. Several validated test methods exist for determining potential inhalability/respirability, *i.e.*, particle size, of a new chemical substance (*e.g.*, OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><d

ates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], ISO 21501-1:2009 [ADDIN EN.CITE <EndNote><Cite><Author>ISO</Author><Year>2009</Year><RecNum>14820</RecNum><DisplayText>[121]</DisplayText><record><rec-number>14820</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046993">14820</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ISO</author></authors></contributors><titles><title>Determination of particle size distribution — Single particle light interaction methods — Part 1: Light scattering aerosol spectrometer</title></titles><pages>https://www.iso.org/standard/42728.html</pages><volume>ISO 21501-1:2009</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], OECD TG 110 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>1981</Year><RecNum>14821</RecNum><DisplayText>[122]</DisplayText><record><rec-number>14821</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047114">14821</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Particle Size Distribution/Fibre Length and Diameter Distributions; Method A: Particle Size Distribution (effective hydrodynamic radius); Method B: Fibre Length and Diameter Distributions</title><secondary-title>OECD Guidelines for the Testing of

Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>13, https://www.oecd-ilibrary.org/docserver/9789264069688-en.pdf?expires=1596047951&id=id&accname=guest&checksum=A9C13F0DFDCF2A5DD4DD39DAC64C47BC</pages><volume>110</volume><dates><year>1981</year></dates><urls></urls></record></Cite></EndNote>], and OPPTS 830.7520 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>14822</RecNum><DisplayText>[123]</DisplayText><record><rec-number>14822</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047315">14822</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Particle Size, Fiber Length, and Diameter Distribution</title><secondary-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</secondary-title></titles><periodical><full-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPPT-2009-0151-0030&contentType=pdf</pages><volume>EPA 712-C-96-037</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]). The studies shown in Table 3 suggest that the total respiratory tract may be affected from surfactants; therefore, inhalable forms (≤ 100 μm) were identified as the most relevant for quantitative inhalation risk assessment. As a practical matter, a particle size cutoff of greater than 1% inhalable particles/droplets by weight (wt%), determined in a well conducted study using a

valid measurement method will generally be considered as triggering a quantitative assessment of inhalation toxicity on a new chemical substance meeting the Surfactant Criteria. EPA will generally assess the potential inhalation toxicity for a new surfactant chemical substance when the manufacture, processing or use results in greater than 1% (by weight) of the surfactant particles/droplets having a particle size of less than 100 μm. This wt% cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2017</Year><RecNum>14823</RecNum><DisplayText>[124]</DisplayText><record><rec-number>14823</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047488">14823</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>3641-3655</pages><volume>82</volume><number>8</number><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>].

If inhalable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information to determine whether a new chemical substance invokes MIEs and cellular level key events. In order to determine the best approach for *in vitro/ex vivo* testing, a pre-notice consultation with EPA is highly encouraged. In general, the testing approach in this tier should include a combination of assays, such as one that measures epithelial lining fluid/cell perturbation or pulmonary surfactant interaction/loss of function, one that measures cell membrane interaction/disruption/penetration), and one that measures loss of barrier integrity or general cytotoxicity (see [REF _Ref46931271 \h * MERGEFORMAT]). *In vitro/ex vivo* eye irritation studies may also be used to demonstrate cell interaction or penetration and general cytotoxicity, and *in vitro* skin irritation/corrosion studies can provide supporting evidence of possible irritant or corrosive effects in the respiratory tract.

For each assay, the comparator substance for the respective subcategory of surfactants should be tested under identical conditions. Further, the particle size distribution data may be used with dosimetry models such as RDDR or MPPD to aid with identifying the regions in the respiratory tract where deposition is expected to occur and the appropriate test concentrations for the *in vitro/ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Notwithstanding the uncertainties with the above assays, each may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE) for the purpose of evaluating the relative potency of the new chemical substance versus the comparator substance. Several investigations have provided insight on approaches for

accomplishing this, although with different assay systems [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In doing so, a weight of scientific evidence evaluation should be performed considering the structural features, physicochemical properties, and assay results on the new chemical substance versus the comparator substance. Based on this evaluation, the most biologically relevant endpoint(s) should be used to calculate a POD. BMD modeling may be applied to derive a $BMCL_{1SD}$ metric, as a possible metric, although the metric of one standard deviation should be used with caution [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2019</Year><RecNum>14825</RecNum><DisplayText>[126]</DisplayText><record><rec-number>14825</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048386">14825</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Transmittal of Meeting Minutes and Final Report for the Federal Insecticide Fungicide and Rodenticide Act, Science Advisory Panel (FIFRA SAP) Meeting held on December 4 and 6, 2018</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>51,https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2018-0517-0030&contentType=pdf</pages><volume>EPA-HQ-OPP-2018-0517</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] . Alternative metrics should be considered, as our understanding evolves for various *in vitro*

assays and endpoints. For example, the pharmaceutical industry has used fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc.*) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Regardless of the metric used, a justification for its selection should be provided. In those situations where data are not amenable to BMD modeling, the *in vitro* concentration tested should be determined based on the expected HEC for the appropriate subcategory (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC.

Given that the understanding of IVIVE is evolving, assay results should be interpreted in a manner consistent with the weight of scientific evidence, as noted above, while recognizing that uncertainties are often dealt with by erring on the side of conservativism. Therefore, the following initial default criteria are proposed for utilizing the assay results, and when possible, the IVIVE estimates. These criteria are consistent with EPA's approach for evaluating non-animal skin sensitization data [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14832</RecNum><DisplayText>[128]</DisplayText><record><rec-number>14832</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596244984">14832</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing (draft for public comment: April 4, 2018)</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and

Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2016-0093-0090&contentType=pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], while recognizing that the weight of scientific evidence may support an alternative interpretation to the default criteria.

The Tier II assays evaluate biologically relevant endpoints representing key events in AOPs relevant to the Surfactant Category. The results of the comparator substance and the new chemical substance in these assays provide a basis for evaluating the suitability of using the comparator substance to evaluate toxicity of the new chemical substance. Consideration should also be given to differences in the specific physicochemical properties influencing inhaled deposition (*i.e.*, MMAD, GSD, and density) between the comparator substance the new chemical. Dosimetry models such as RDDR and MPPD can be used to inform these comparisons.

If comparable toxicity is observed between the comparator substance and the new chemical substance in the Tier II assays, the POD_{HEC} from the comparator substance may be appropriately used as a toxicological analogue for quantifying the MOE. If calculated risk is acceptable stop at Tier II, otherwise proceed to Tier III.

If lower toxicity is observed for the new chemical substance versus the comparator substance in the Tier II assays, then these data should be used to determine if a modified POD_{HEC} can be quantified for the new chemical substance. If this is possible, the modified POD_{HEC} for the new chemical substance should be used for quantifying the MOE. If calculated risk is acceptable, then stop at Tier II. However, if it is not possible to calculate a modified POD_{HEC}, then the comparator substance POD_{HEC} could be used as a worse-case toxicological analogue for risk assessment. If no acceptable risk can be calculated, proceed to Tier III.

If greater toxicity is observed with the new chemical substance versus the comparator substance in the Tier II assays, suggesting risks would be identified as unacceptable, proceed to Tier III. Alternatively, there may be scientifically justified reasons for an alternative interpretation, which should be clearly articulated with the weight of scientific evidence evaluation. Otherwise, it may be necessary to proceed to Tier III.

If the results from the Tier II assays are equivocal (*i.e.*, they do not demonstrate comparable or lower toxicity of the new chemical substance versus the comparator substance), and there is no clear rationale or explanation, then proceed to Tier III testing because the data are too uncertain to make a reasoned evaluation on the potential health risks, following potential inhalation exposures.

Tier III – 3D Human Airway Models/PCLS Assay

Several testing options are available for evaluating tissue and organ level key events in an AOP relevant to the Surfactant Category. The test system employed should focus on evaluating effects

in the respiratory tract at the predicted sites of deposition (*e.g.*, ET, TB and/or PU regions), based on the particle size distribution data generated under Tier I and using RDDR or MPPD modeling. A justification for using a system(s) should be provided and may be discussed with EPA as part of a pre-notice consultation. Representative test systems include those listed in [REF _Ref46931271 \h * MERGEFORMAT].

Based on the results of the 3D-construct and/or PCLS testing, IVIVE may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[112]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-

title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33, https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use or if the Tier III test data are not amenable for developing a POD_{HEC}, then proceed to Tier IV.

Tier IV – *In vivo* studies

Strategic *in vivo* testing may be considered as a last resort to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the comparator substances in a subcategory has representative toxicological properties to the new chemical substance, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving modified POD_{HEC}s. A pre-notice consultation meeting with EPA is strongly encouraged prior to initiating any vertebrate animal testing. This point is especially important because TSCA section 4(h)(3) indicates that any person developing information for submission under TSCA section 5 on a voluntary basis shall first attempt to develop the

information by means of an alternative test method or strategy identified by EPA before conducting new vertebrate animal testing [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>].

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with epithelial lining fluid/pulmonary surfactant and/or disrupting cellular membranes and epithelial cytotoxicity. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests should be considered:

- Step 1: OECD TGs 433, 436, and 403 address acute inhalation toxicity testing. OECD TG 433 is based on evident clinical signs of toxicity rather than death as an endpoint

(refinement) and TG 436 uses fewer of animals (reduction), and therefore, they should be considered before TG 403. Any protocol modifications should be discussed with EPA during a pre-notice consultation meeting.**

- Step 2: 5-Day inhalation study with a 14-day observation period** to address progression/resolution of effects. The OECD TG 412 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14828</RecNum><DisplayText>[129]</DisplayText><record><rec-number>14828</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048957">14828</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>28-day (subacute) inhalation toxicity study</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>23, https://doi.org/10.1787/9789264070783-en</pages><volume>412</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be used, but the exposure duration should be 5 days.

**Modifications may include pulmonary function testing (if measurable), analysis of BALF, LDH release, complete histopathological analysis of the respiratory tract and blood oxygen (pO_2) content. OECD TG 412 and OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum>

<DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>2001</Year><RecNum>14826</RecNum><DisplayText>[130]</DisplayText><record><rec-number>14826</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048712">14826</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Alarie, Y.</author><author>Nielsen, G.D.</author><author>Schaper, M.M.</author></authors><secondary-

authors><author>Spengler, B.</author><author>Samet, J. M.</author><author>McCarthy, J.F.</author></secondary-authors></contributors><titles><title>Animal Bioassays for Evaluation of Indoor Air Quality</title><secondary-title>Indoor Air Quality Handbook</secondary-title></titles><pages>23.21-23.49.</pages><dates><year>2001</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill</publisher><urls></urls></record></Cite></EndNote>].

The results of the *in vivo* testing should be used for reassessing and recharacterizing the risks of the new chemical substance.

CONCLUSIONS

The overall objective of this investigation was to develop a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. This investigation developed physical-chemical properties, *i.e.*, the Surfactant Criteria, assessors and product stewards can use for determining whether a new chemical substance can be considered a surfactant. Further, properties and characteristics are provided to divide the Surfactant Category into sub-categories for nonionic, anionic, and cationic surfactants, which is important from a toxicological perspective. A systematic literature search and review were conducted to identify data to define a Surfactant Category and substances from which PODs were identified from inhalation toxicity studies. To facilitate chemical comparisons, animal toxicity studies that could be used to derive PODs for risk assessments were identified for at least one chemical substance for each sub-category and converted to HECs using established methods developed by EPA.

Finally, a tiered-testing strategy for generating *de novo* data for new surfactant substances is provided that integrates a variety of currently available NAMs using an AOP framework. The use of this tiered-testing strategy will inform the available data on surfactants and provide greater confidence in the use of non-vertebrate testing approaches for assessing the potential risks of new chemical substances. It also offers advantages to regulators, the regulated community, and consumers because: 1) integrating NAMs into a category testing approach supports EPA, TSCA and product stewardship goals of reducing and replacing vertebrate animal testing; 2) decision analysis for higher tiered testing takes into consideration mechanistic responses, dosimetry, and exposure information; and 3) it encourages development of mechanistic data to advance the understanding of the potential inhalation toxicity of surfactants, which will drive the development of newer and safer chemistries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information file contains the following:

Section 1. Systematic Literature Review

Section 2. RDDR Modeling Outputs

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Author Contributions

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Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

Disclosures: TS, AMJ, KS, WI, and TRH are employed by the federal government. MPH, WK, AMK, SM, LJ, JLR, AT, and RT are employed by companies that manufacture, process, and/or use surfactants. RAB and SOS are employed by a company that represents companies that manufacture, process, and/or use surfactants. PDM and SDS work for a company that received contract funding from companies that manufacture, process, and/or use surfactants. MO and JM

work for a company that receives contract funding from the federal government. AJC and MS are employed by a company whose mission is to advance animal-free testing approaches that protect human health and the environment.

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Surfactants Category: The Application of a New Approach Methodology (NAM) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

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KEYWORDS: Inhalation, Surfactant, New Approach Methodologies, Lung Toxicity, Risk Assessment

ABSTRACT

The Toxic Substances Control Act (TSCA) requires anyone who plans to manufacture (including import) a new chemical substance for a non-exempt commercial purpose to provide the U.S. Environmental Protection Agency (EPA) with a premanufacture notice (PMN) prior to commercialization. Surfactants are a class of chemical substances used in a variety of industrial operations, occupational settings, and in consumer products. Their uses in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to humans. While TSCA requires submission of any existing toxicity data, it does not require generation of toxicity data for the purpose of, or prior to, submitting a PMN. TSCA requires EPA to review the PMN to determine whether the new chemical substance presents an unreasonable risk of injury to human health or the environment and mandates that EPA reduce or replace vertebrate animals

in testing, to the extent practicable and scientifically justified. EPA therefore relies on several approaches that do not rely on *de novo* toxicity testing. Analogue read-across, in which toxicity data for a chemical of similar structure and activity are used to assess the new chemical, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) have been used by EPA for decades to assess new chemical substances. This investigation was conducted to identify surfactant chemicals with toxicity data relevant for use in conducting a quantitative human health risk assessment for new surfactant substances and to define a TSCA New Chemical Category for surfactants. Category boundaries, which are defined, toxicological analogues suitable for conducting 'read-across' hazard assessment (*i.e.*, hazard identification and dose-response analysis) are identified and a tiered-testing strategy aimed at using new approach methodologies (NAMs) to reduce or replace animal testing is outlined. This tiered strategy to defining and evaluating the Surfactant Category provides a pragmatic and scientifically defensible approach to facilitate EPA's review of PMNs for new surfactants and a strategic testing approach that provides the

data needed to conduct or refine surfactant risk assessments while also meeting the requirements of TSCA to reduce vertebrate testing.

INTRODUCTION

The Toxic Substances Control Act (TSCA) was amended in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182). The amended TSCA included substantial changes to EPA's authorities and responsibilities, including requirements on EPA to make a determination regarding sufficiency of information, environmental releases and human exposure, and unreasonable risks. The amended TSCA also included provisions mandating EPA to "reduce and replace, to the extent practicable, [and] scientifically justified" the use of vertebrate animals in the testing of chemicals substances. Specifically, TSCA section 4(h) charges EPA with encouraging and facilitating –

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or

better scientific quality and relevance that will support regulatory decisions under TSCA;

(2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and

(3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle

concentration (CMC). These substances are commonly used in industrial processes, occupational settings, and in consumer products (*e.g.*, household cleaning products, personal care products, *etc.*) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The widespread use of surfactants provides opportunities for releases and exposure to human or environmental receptors. The inherent properties of surfactants may induce toxicity if exposures can interfere with biological surfactants or tissues. Certain surfactants are commonly used in a laboratory setting to disrupt cell membranes and denature proteins, which demonstrates the inherent hazards of surfactants. For example, sodium dodecyl sulfate (SDS; Chemical Abstracts Service Registry Number (CASRN) 151-21-3), a strong anionic surfactant, is used at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol (CASRN 9002-93-1), a mild nonionic surfactant, at concentrations up to 1% disrupt cell membranes, while preserving proteins for isolation [1].

Hazard concerns for surfactants historically focused on their observed environmental effects and potential toxicity to aquatic organisms based on “down the drain” releases and/or presence in effluent from wastewater treatment facilities [2].The EPA has established chemical categories for nonionic, anionic, and cationic (quaternary ammonium) surfactants based on environmental toxicity concerns [3]. Surfactants may pose a potential hazard to humans, depending on their use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. Mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to “readily penetrate the sandwiched aqueous and lipid barriers of the cornea” [4].

Depending on the conditions of use, the potential for inhalation exposures to workers and/or consumers warrant consideration in quantitative risk assessments. Surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and interfere with the natural pulmonary surfactants and result in reduction in the oxygen

content of arterial blood due to impaired gas exchange in the pulmonary region, increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis [5-7]. The chemical category boundary for surfactants that may have the potential to present an inhalation hazard has not been previously defined. The toxicity of surfactants by inhalation exposure can vary over several orders of magnitude, based on their chemical properties, although differences in exposure conditions are an important confounder to consider in cross category comparisons. For example, among the available data, a lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) was determined for octylphenoxypolyethoxyethanol, a nonionic surfactant, in a 14-day whole body study[8, 9] while a LOAEC of 0.08 mg/m^3 in a 4-week nose-only study [10] was observed for didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5), a cationic surfactant and biocide.

The objectives of the present investigation were to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify

inhalation toxicity studies on surfactants that may be used to inform inhalation risk assessments; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing; and (4) establish a tiered-testing strategy that uses NAMs to evaluate new chemistries in the Surfactant Category.

MATERIALS AND METHODS

Systematic Literature Review

Two literature searches were performed, an initial search from 1950 through November 2016 and a supplemental search up to April 2018. The details of these searches, including the search strategies, search terms, search results and Population, Exposure, Comparison, and Outcome (PECO) criteria used for reviewing the relevance of the identified studies to this evaluation are provided in the Supporting Information file at "Section 1 Systematic Literature Review". These searches were conducted with the primary objective of identifying studies that evaluated the toxicity of surfactants in the respiratory tract of humans or laboratory animals, and at the cellular level in *in vitro* and *ex vivo* studies. In addition, these searches were used to identify potential NAMs that

could inform a tiered-testing strategy for general surfactants that reduces or replaces the use of vertebrate animals in regulatory testing.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The methods for assessing risks of new chemical substances under TSCA have been developed using science-based approaches, scientific peer review, and refinement of the approaches. EPA conducts risk assessments following the four-step process articulated by the U.S. National Research Council (NRC) in 1983 [11] and reaffirmed several times since its initial release [12, 13]. This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the adverse health or environmental effects, or hazards, that can be caused by exposure to a chemical substance. The dose-response analysis assesses the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects. The exposure assessment characterizes human or

environmental exposures, including the magnitude, frequency, and duration, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure components to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these risk assessment components, including the level of detail and complexity of quantitative aspects, may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc.* are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations [11], companies are required to submit a PMN along with available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or

control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances.

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives hazard data for new chemical substances. An analysis of toxicity data submitted to EPA from 2004 through 2012 for new chemical substances found that only about 15% of the PMN submissions included health hazard data; the majority of that information was for acute toxicity (*e.g.*, 24-hour dermal toxicity study with a 14-day post-administration observation period) and irritation (*e.g.*, 4-hour dermal irritation/corrosion with a 14-day post-administration observation period or 24-hour eye irritation/corrosion with a 21-day post-administration observation period) in laboratory animals. TSCA provides EPA with the authority to require the generation and submission of additional data when the information included with the PMN— coupled with that available to EPA

risk assessors from predictive modeling, read-across, internal archives, *etc.* —is insufficient to permit a reasoned evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data, EPA has, for decades, employed a number of approaches that do not rely on *de novo* toxicity testing. These approaches include computational toxicology (*e.g.*, predictive models and expert systems), analogue[1] read-

[1] In the context of this article, an analogue is a chemical substance identified based on its physicochemical and toxicological properties, as one that has undergone evaluation, as stated above, and determined to be an acceptable toxicological analogue for read across to the new chemical substance. An analogue may be directly used in read-across for informing a quantitative risk assessment on a new chemical substance.

across wherein available toxicity data for a chemical of similar structure and activity are used to assess the new chemical substance lacking data, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) [12-14]. The integration of these methods with NAMs to advance testing strategies has been recognized by Dellarco *et al.* [15] and is consistent with the vision articulated in the 2007 report by the NRC in "Toxicity Testing in the 21st Century: A Vision and Strategy" [16]. EPA defines NAMs "as a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals" [17]

Dose-Response Analysis

In the absence of test data on new chemical substances, EPA relies on read-across methods using an analogue or a category of analogues in the absence of test data on the new chemical substance to identify hazards and conduct dose-response analysis to identify a point of departure (POD), *i.e.*, a dose or concentration that marks the

beginning of a low-dose extrapolation. Toxicity data for analogues are used to identify a POD, such as a no observed adverse effect (concentration) level (NOAE(C)L) or lowest observed adverse effect (concentration) level (LOAE(C)L, for assessing risks of the new chemical substance. This POD can also be the lower bound on dose (or concentration) for an estimated incidence or a change in response level calculated by a dose-response model such as those available in EPA's benchmark dose software (BMDS), *e.g.*, the BMCL for an observed incidence or change in level of response [18]. EPA's current chemical categories document on surfactants entitled "TSCA New Chemicals Program (NCP) Chemical Categories" [3] includes information for anionic, nonionic, and cationic surfactants; however, these were previously developed and defined only on environmental toxicity considerations.

EPA has also developed guidance to improve the science underlying the animal-to-human uncertainty factor and provides generalized procedures for deriving dosimetric adjustment factors (DAFs) to perform interspecies extrapolation [19, 20]. Application of DAFs to the animal airborne exposure values yields estimates of the concentration that

would result in the same concentration to humans, that is, the human equivalent concentration (HEC). Application of a DAF in the calculation of an HEC is considered to address the toxicokinetic (TK) aspects, but not the toxicodynamic (TD) component, of the animal-to-human uncertainty factor (UF) (*i.e.*, to estimate from animal exposure information the human exposure scenario that would result in the same dose as achieved in the animal to a given target tissue) [19]. This operational derivation of a DAF involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle, reactive gas, or volatile organic compound) coupled with consideration of the location and type of toxic response. These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (*e.g.*, to a weekly average based on number of h/d and d/w).

For interspecies extrapolation of particle exposures, the Regional Deposited Dose Ratio (RDDR) model developed by EPA can be used to derive a DAF. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of

interest (RDD_A) to that of humans (RDD_H) [20]. EPA's RDDR model allows calculation of RDDR estimates in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic [ET], tracheobronchial [TB], pulmonary [PU], thoracic [TH], total respiratory tract [RT] and extra-respiratory [ER] regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (*i.e.*, the Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD, and density), and species-specific parameters for both animals and humans including ventilation rates and regional surface areas of the respiratory tract. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The DAF is then applied to the duration-adjusted POD to arrive at the HEC of the POD (POD_{HEC}). The EPA's RDDR model was used herein to calculate HEC values from the aerosol exposures to laboratory animals available for each of the surfactant classes.

After an analogue(s) is identified, the strengths, limitations, and uncertainties associated with the use of the substance(s) to predict the hazards for the new chemical substance under evaluation are considered when deriving a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant UFs to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less-than-lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL to a NOAEL [19, 21]. EPA prefers using existing information to develop data-derived extrapolation factors (DDEFs) or chemical specific adjustment factors (CSAFs) rather than relying on default values [21]. This investigation includes several approaches to derive DDEFs for use in assessing new surfactant chemical substances.

Exposure Assessment

In assessing new chemical substances, generally new chemical substances do not have occupational exposure monitoring data or consumer exposure data; therefore, EPA typically evaluates occupational exposures first, given that these represent the highest exposure estimates. Therefore, this evaluation focused on occupational exposures, recognizing that consumer exposures would also be considered, if applicable. EPA develops exposure estimates for workers using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER) model. ChemSTEER estimates exposure as daily acute potential dose rates (PDRs) or lifetime average daily doses (LADDs). The PDR represents average exposure over an 8-hour workday, whereas the LADD estimates long-term exposures to the chemical substance and is averaged over a lifetime exposure of 75 years. The PDR, an initial conservative exposure estimate, is considered to be the more appropriate dose-metric for estimating risks to surfactants because surfactants are surface-active at the point of exposure and effects in the respiratory tract occur rapidly following exposure. This assumes that neither the chemical nor its damage accumulate or distribute to systemic compartments. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR

values are 1.875 mg/kg-bw/day for inhalable aerosols or 0.625 mg/kg-bw/day for respirable aerosols as shown in Table 1 [22].

Table 1. Default values used for calculating the daily acute potential dose rate (PDR).

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day

		Body weight (BW)	BW (0 ≤ BW)	80 kg-bw	kg-bw

[a] Cm may also be adjusted for the mass concentration of the chemical with a permissible exposure limit (PEL) in air (based on the U.S. Occupational Safety and Health Administration [OSHA] PEL – time-weighted average [TWA]; where: KCk = the mass concentration limit of total particulate in air (mg/m^3) with a default of 15 mg/m^3 for inhalable and 5 mg/m^3 for respirable, Ys= the weight fraction of chemical in particulate (0 < Ys ≤ 1), Ypel=the weight fraction of chemical or metal in particulate with a known PEL (0 < Ypel ≤ 1) using the following equation: Cm = KCk × Ys/Ypel

The PDR is calculated using an exposure regimen for a default worker of 8 hours/day and 5 days/week, unless chemical-specific manufacture, processing or use information are provided in the PMN. The exposure conditions in laboratory animal studies often do not reflect occupational exposure scenarios; therefore, a duration adjustment and a DAF (*i.e.,* RDDR value) are applied to the POD to derive HECs for exposed human populations according to Agency methods [20]. Therefore, the interspecies extrapolation is performed using particle deposition models that adjust for the aerodynamics of the given particles in the different airway architecture between the species and using species-specific physiologic parameters such as ventilation. The occupational exposure is characterized with human ventilation rates during exertion (work) and exposure durations appropriate to the specific occupational setting and chemical use scenario.

Risk Characterization

Risk characterization is the final, integrative step of risk assessment. EPA's Risk Characterization Policy defines risk characterization as the integration of information from the hazard and exposure components of the risk assessment into an overall conclusion about risk that is complete, informative, and useful for decision-making. The risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks [23]. As described in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized and the level of information contained in each risk characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic

exposures. The MOE is the HEC derived from a POD for a health endpoint (from hazard assessment) divided by the exposure concentration for the scenario of concern (from exposure assessment). The calculated MOE is compared with a benchmark MOE to evaluate whether there is an adequate margin between human exposure estimates and the HEC. When the MOE is less than the benchmark MOE, there is a possibility of human health risks. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios.

In summary, in developing a risk assessment for new chemical substances under TSCA section 5, EPA uses empirical data or analogues, to identify a POD(s) and to develop an exposure estimate for use in the evaluation. The hazard assessment in combination with the exposure assessment is used to calculate an MOE, which is compared to the benchmark MOE to identify potential risks. The risk characterization is used to inform the TSCA "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

An initial search of PubMed identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles that met the PECO criteria were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc.*, and were included for full text review. Of the 60 articles evaluated through full text screening, 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search of PubMed and Embase, 1247 articles (combined) were identified. Following title and abstract screening, 1217 of these articles were excluded because they did not meet the PECO criteria, whereas 25 met the PECO

criteria and were selected for full text review. An additional 10 studies that met the PECO criteria were found by additional hand searching) and were selected for full text screening, which resulted in 35 articles that were identified for review; ten articles were deemed irrelevant and excluded. A total of 25 articles were identified from both searches, one was excluded because it was in a foreign language and the remaining 24 articles are summarized in Table 8 in the Supporting Information file at "Section 1 Systematic Literature Review".

The information identified in the systematic review was used to determine Category Boundaries and subcategories, to summarize the health effects of surfactants under the section on Hazard Identification, and to identify potential NAMs for use in the Tiered-Testing Strategies.

Category Boundaries

The following structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and

UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) [24-26]:

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance is capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test concentration of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less.

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

The Surfactants Category is further defined into three general subcategories including nonionic, anionic, and cationic substances. Amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, anionic and cationic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging from 6.6 to 7.1 [27-29]. The pKa for each component of an amphoteric surfactant should be evaluated within this pH range and the assessment should be conducted on the predominant components. The non-ionized fraction for acids/bases is calculated as follows:

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pH-pKa}})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pKa-pH}})$$

Where the pH represents the physiological pH in the lung lining fluid (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine).

Nonionic surfactants are identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. For example, octylphenoxypolyethoxyethanol, a common nonionic octylphenol EO surfactant, and Polysorbate 80 (or Tween 80; CASRN: 9005-65-6), another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table 2. The surface tensions of octylphenoxypolyethoxyethanol and Polysorbate 80 range from 30-31 mN/m to 37.96 mN/m, respectively (Table 2) [30].

Anionic surfactants are identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). An example anionic surfactant, SDS, has a reported surface tension of 35 mN/m (Table 2).

Cationic surfactants are identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). Benzalkonium chloride (BAC; CASRN 8001-54-5) and didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5) are representative members of this subcategory, with surface tensions of 37 mN/m and 25.82 mN/m (Table 2), respectively. It is noted that BAC and DDAC also possess biocidal properties.

Typical commercial surfactants (nonionic, anionic, and cationic) are non-volatile[3] liquids or solids. This category framework focuses on exposure *via* aerosol forms (*i.e.*, both airborne droplets and solid particles, including the hygroscopic variety) of these surfactants. While the commercial use of volatile surfactants is unlikely, it should be noted

[3] Volatility is considered as part of the ChemSTEER modeling, wherein a vapor pressure of 1.3×10^{-04} kPa is the cutoff for gases/vapors.

that this framework is not applicable to any substances that qualify as surfactants and are volatile under the conditions of use.

Table 2. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cationic Subcategorization.

Nonionic Surfactants					
Chemical Name in Text	Other Relevant Names	Criteria 1		Criteria 2	Criteria 3
		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol Defomaire Alevaire Tyloxapol CASRN: 25301-02-4	CAS Name: formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol	multiple octyl phenol groups	multiple polyoxyethylene (9) units	~37 mN/m at 5 g/L (0.5 wt%) and 25°C* [31]	0.038 g/L or 0.0038 wt% [31]

octylphenoxypolyethoxyethanol CASRN: 9002-93-1	Triton X-100 Octoxynol 9 octylphenol ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	octylphenol group	polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C* [31]	0.17 g/L or 0.017 wt% [31]
polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$) CASRN: 9004-98-2	oleyl ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	oleyl group	polyoxyethylene (10) unit	35.17 mN/m at 4×10^{-5} M (0.028 wt%) and 25°C* [32]	4×10^{-5} M or 0.028 wt % at 25°C [32]
polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$)	polyoxyethylene (10) lauryl ether	dodecyl group	polyoxyethylene (10) unit	C12E9: 36 mN/m (concentration not reported) at 23°C*	12.7×10^{-6} M or 0.0008 wt% at 30°C [34]

CASRN: 9002-92-0	CAS Name: poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-			C12E12: 32 mN/m (concentration not reported) at 23°C* [33]	Also, C12E9 at 1×10^{-6} M at 23°C and C12E12 at 1.4×10^{-6} M at 23°C [33]
Polysorbate 20 (Tween 20) CASRN: 9005-64-5	polyoxyethylene (20) sorbitan monolaurate CAS Name: sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	dodecanoyl group	sorbitan polyoxyethylene (20) unit	38 mN/m at 8.04×10^{-5} M (0.001 wt%) and 21°C* [35]	8.04×10^{-5} M or 0.001 wt% at 21°C [35]
Polysorbate 80 (Tween 80) CASRN: 9005-65-6	polyoxyethylene (20) sorbitan monooleate CAS Name: sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	octadecenoyl group	sorbitan polyoxyethylene (20) unit	37.96 mN/m at 5 g/L (0.5 wt%) and 30°C [30]	1.5×10^{-5} M or 0.002 wt% at 25°C [36]

Poloxamer 188 CASRN: 691397-13-4	CAS Name: oxirane, 2-methyl-, polymer with oxirane, triblock	polyoxypropylene (27) unit	two polyoxyethylene (80) units	~42-44 mN/m at ~0.5 wt% and 36°C [37]	4.8×10^{-4} M or 0.4 wt% at 37°C [38]
N,N-dimethyl-dodecylamine-N-oxide (C_{12}AO)*** CASRN: 1643-20-5	lauryl dimethylamine oxide CAS Name:1-dodecanamine, N,N-dimethyl-, N-oxide	dodecyl group	amine oxide unit	34.1 mN/m at 1 g/L (0.1 wt%) and 20°C [39]	1.7×10^{-3} M or 0.039 wt% [40] 1×10^{-5} M to 5.5×10^{-5} M or 0.0002 to 0.001 wt% at 25°C [41]
Anionic Surfactants					
Chemical Name in Text	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**
		Hydrophobic group(s)	**Hydrophilic group(s)**	**Surface Tension**	**Critical Micelle Concentration (CMC)**
sodium dodecyl sulfate (SDS) CASRN: 151-21-3	CAS Name: sulfuric acid monododecyl ester sodium salt (1:1)	dodecyl group	sulfate group	35 mN/m at 0.29 wt% and 20°C [42]	8.25×10^{-3} M or 0.24 wt% at 20°C [41]

oleoyl sarcosine CASRN: 110-25-8	CAS Name: glycine, N-methyl-N-((9Z)-1-oxo-9-octadecen-1-y	oleyl group	carboxylic acid anion	31.91 mN/m at 0.1 wt% and 19.9°C** [43]	2.6×10^{-3} wt% and ~25°C ** (temperature not reported, assumed to be room temperature) [44]
sodium lauroyl sarcosinate CASRN: 137-16-6	CAS Name: glycine, N-methyl-N-(1-oxododecyl)-, sodium salt (1:1)	lauryl group	carboxylic acid anion	40.5 mN/m at 2 wt% and 20°C [45]	8.0×10^{-2} wt% and ~25°C (temperature not reported, assumed to be room temperature) [44]
dioctyl sulfosuccinate sodium salt (DOSS) CASRN: 577-11-7	dioctyl sodium sulfosuccinate CAS Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt	two 2-ethyl hexyl groups	sulfosuccinate group	<28 mN/m at 0.5 vol% and 25°C* [46]	6.8×10^{-4} M or 0.03 wt% at 25°C [41]
Cationic Surfactants					

Chemical Name in Text	Other Relevant Names	Criteria 1		Criteria 2	Criteria 3
		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
benzalkonium chloride (BAC) CASRN: 8001-54-5	CAS Name: quaternary ammonium compounds, alkylbenzyldimethyl, chlorides	alkyl chains are C12, C14, C16 and C18 and benzyl group	quaternary nitrogen	37 mN/m at concentrations greater than about 4×10^{-4}M and 25°C* [47]	C12: reported values range from 2.3 - 8.5×10^{-3} M or 0.078 - 0.29 wt% at 25°C C14: 3.7×10^{-4} M or 0.014 wt% and ~25°C (temperature not stated; assumed to be room temperature) C16: 4.2×10^{-5} M or 0.0016 wt% at 23°C C18: reported values range from

					7.1 - 8.5×10^{-6} M or 0.0003 - 0.00036 wt% at 23°C [41]
didecyldimethyl ammonium chloride (DDAC) CASRN: 7173-51-5	CAS Name: 1-decanaminium, N-decyl-N,N-dimethyl-, chloride (1:1)	decyl groups	quaternary nitrogen	25.82 mN/m at 1 g/L (0.1 wt%) and 20°C [48]	0.39 g/L or 0.039 wt% at 25°C [48]

*Not all of the surface tension measurement references identified are run at exactly 20°C, but they are sufficiently close (within 5°C) so as not to affect the measurement. In addition, several measurements were run at 0.1% instead of the recommended 0.5%. Increasing the concentration to 0.5% is likely to lower the surface tension.

**Carboxylic acid compounds, such as oleoyl sarcosine, have a carboxyl group pKa value of ~5, thus at physiological pH values maintained near 7 in the lung, the carboxyl group will be 99% in the anionic form according the Henderson-Hasselbalch equation. Since sodium is the major cation in mammalian body fluids (~145 mM), the use of the sodium oleoyl sarcosine surface tension value is appropriate for its characterization.

***Amphoteric: At pH 7, 90% expected to be nonionic; only small amount cationic.

Hazard Identification

There is concern for dysfunction of mucus, epithelial lining fluid, and natural surfactant lining in the various regions of the respiratory tract from inhalation of surfactants. There is also evidence that some surfactants or similar structures may also interfere with the cell membrane of the epithelium in these same regions [49, 50]. This effect on cell membranes is apparent from data on numerous surfactants indicating irritation to the skin and eye, as noted below. The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in both human volunteers and in laboratory animals [51, 5-7]. The respiratory tract responses to inhaled surfactant aerosol is thought to be in proportion to the exposure concentration and duration, but available data on acute and repeated-dose effect levels are limited within each subcategory, which limits establishing a correlation between chemical properties and toxicity due to exposure methods (*e.g.,* generated aerosol droplet size).

Nonionic Surfactants

In vivo studies

Several studies were identified for the nonionic siliconized superinone respiratory detergent, 4-(1,1,3,3-tetramethylbutyl)phenol polymer with formaldehyde and oxirane (CASRN 25301-02-4; commonly known as Defomarie, Alevaire, and Tyloxapol). Healthy human volunteers demonstrated significantly decreased respiratory compliance following acute inhalation of Defomaire [51]. An increased minimum surface tension due to detergent was shown to be dose-dependent, using pulmonary surfactant extracted from dogs with the nonionic surfactant tyloxapol (Alevaire) *in vitro* [7]. However, *in vivo* exposure of dogs to Alevaire (8-hour aerosol exposure; vehicle, particle size and distribution, and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension). The results did not support the dose-dependence of the effect and indicated that small amounts of detergent in the lungs may not detectably alter the surface tension-surface area relationship and that alteration of surface tension is unlikely to occur during reasonable use although there is considerable uncertainty regarding the internal dose achieved [7].

Inhalation studies using dogs and/or sheep exposed to nonionic surfactant, tyloxapol, resulted in reduced oxygen content of arterial blood due to impaired gas exchange in the lung, increased pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) [5-7]. In the study by Modell *et al.* (1969) [7], no gross pathology differences were seen in detergent-exposed versus control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl) since these effects were not observed in lungs treated with a less dense aerosol. Normal appearances were observed in the remaining areas of the lungs.

In rodents, irritation and inflammatory effects in the entire respiratory tract have been observed with varying degrees of severity. Acute inhalation exposure *via* nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/L (5,100 mg/m^3) with an MMAD of 2.2 µm and a GSD of 2 to Sorbitan monolaurate, ethoxylated

(CASRN 9005-64-5), a chemical not irritating to the skin or eyes [52], did not result in an increase in mortalities, clinical signs, or abnormalities in the gross pathology [53]. A respiratory irritation study using plethysmography was performed on a mixture containing octylphenoxypolyethoxyethanol [9], which can be severely irritating to the skin and eyes, in male Webster mice exposed for 3 hours to concentrations of 12, 22, 51, 118, and 134 mg/m^3 with 30-60 minutes recovery time (MMAD and GSD not provided). Signs of pulmonary irritation were observed in animals at the two highest concentrations as indicated by a decrease in respiratory frequency (33-58% decrease); this response was preceded by an increase in respiratory frequency (11-12.5% increase) at the highest three concentrations without an increase in gross lung abnormalities, pulmonary edema, or lung weight [54]. An acute inhalation exposure study in Syrian hamsters exposed to 3.0 mg/L of octylphenoxypolyethoxyethanol with varying exposure durations showed that lung deposition directly corresponded to mortality with an LD_{50} of 1300-2100 µg with an MMAD of 1.47 µm and a GSD of 1.84 [55]. The deaths in these animals were attributed to severe laryngeal edema and ulcerative laryngitis while the lower airways in these animals were relatively free of

serious pathologies which likely indicates limited deposition to the lower airways in this study. The authors hypothesized that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the chemical resulted in a large concentration on the laryngeal mucosa, though laryngeal deposition is typically a function of aerodynamics. In the only 2-week whole-body inhalation study for nonionic surfactants, male and female Sprague-Dawley rats were exposed to 5.3 and 10.3 mg/m^3 (5/sex/dose; MMAD 1.8 µm, GSD 1.8) octylphenoxypolyethoxyethanol for 6 hours/day, 5 days/week [9]. Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge; a LOAEC of 5.3 mg/m^3 was identified.

Mechanistic studies

In vitro studies of surfactant on cell membranes have provided evidence of possible modes of action (MOAs). Warisnoicharoen *et al.* (2003) [56] evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$; CASRN 9004-98-2), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$; CASRN 9002-92-0), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) on submerged cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay by exposing the cells to 0.1mL of the serially diluted microemulsion (particle size not reported) for 30 minutes followed by a 60 minute incubation with a MTT solution. All surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg *et al.* (2019) [57] evaluated the cytotoxic activity of the three nonionic polymeric surfactants Polysorbate 20 (CASRN 9005-64-5), Polysorbate 80 (Tween 80)and Poloxamer 188 (CASRN 691397-13-4), which are commonly used in

formulations of nebulized pharmaceuticals to prevent protein agglomeration, in a BEAS-2B human bronchial epithelial cell model using an innovative air-liquid interface (ALI) method of exposure with a nasal spray system (MMAD and GSD not provided). In this study, the ALI results were compared to the classical submerged cell culture or liquid/liquid (L/L) model. The study measured the release of lactate dehydrogenase (LDH), an intercellular enzyme present in the cytoplasm, indicative of the loss of membrane integrity. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method; however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to a lesser extent, Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any *in vitro* cytotoxicity.

The available *in vitro* and *in vivo* data indicate inconsistency in respiratory toxicity among nonionic surfactants; however, the degree to which the variation is due to

experimental design or bioactivity of the surfactant is not discernible from these data. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties, such as surface tension or CMC. Similarly, the examination of the relationship between chemical properties of nonionic surfactants and eye irritation has not established that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths can be used to predict eye irritation potential across the nonionic surfactant subcategory [58]. However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when surface tension was measured under dynamic conditions (0.24, 1, and 4 bubbles/second). Whether this chemical property similarly predicts potency of nonionic surfactants for respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

In vivo studies

Two acute inhalation toxicity studies were identified for anionic surfactants, both demonstrated high toxicity *via* the inhalation route. Oleoyl sarcosine (CASRN 110-25-8), irritating to the skin and damaging to the eye [59], was evaluated in a 4-hour nose-only inhalation study in male and female Sprague-Dawley rats at concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L (300, 600, 2,200, 3,700 mg/m^3). The MMAD and GSD were not reported. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For sodium lauroyl sarcosinate (CASRN 137-16-6), irritating to the skin and corrosive to the eye (undiluted) [60], 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L (50, 500, 1,000, and 5,000 mg/m^3) with a MMAD of 4.4, 2.9, 3.7, and 6.0 µm; and GSD of 2.7, 3, 4.2, and 2.9, respectively. Additionally, 5 female rats were exposed to 1.1 or 5.5 mg/L with a MMAD 3.7 or 6.0 µm and GSD of 4.2 or 2.9, respectively [60, 61]. The 5 mg/L dose resulted in fatality in all 10 animals (males and females) tested within 1-2 h of dosing and the 0.5 mg/L dose resulted in fatality for 4/5 of the males and exposure to 1 mg/L resulted in fatalities for the 10 animals (males and females) within 1-2 days of exposure. Males exposed to 0.05 mg/L did not demonstrate any adverse clinical signs

or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in males and females receiving concentrations of ≥ 0.5 mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Repeated-dose inhalation studies were identified for oleoyl sarcosine, and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (6 hours/day, 5 days/week; Organization for Economic Cooperation and Development [OECD] Test Guideline [TG] 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L (0, 6, 20, or 60 mg/m^3). The particle exposure MMAD was 1.11, 1.15, or 1.22 μm, GSD 1.68-2.57, and density 0.79 g/cm^2 for 6 hours/day, 5 days/week in 10% ethanol [62]. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes were observed in male animals at the high exposure concentration. In female animals of the mid-concentration exposure group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals at the mid and high concentrations, which is associated with severely irritating substances. All test concentrations caused effects at

several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the alveoli walls and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The LOAEC was 0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Dioctyl sulfosuccinate sodium salt (DOSS; CASRN 577-11-7) was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex). Rats were exposed to an aerosol of a product containing 0.0042 mg/L (4.2 mg/m^3) DOSS, for 4 hours a day, 5 days a week (as reported in a secondary source; exposure details, MMAD, and GSD not reported) [63]. There were no statistically significant differences in exposed and control groups for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood as indicated by elevated erythrocytic values (not otherwise specified) at 7 weeks and depressed mean corpuscular hemoglobin concentration values at 13 weeks

in male rats. In females, depressed serum glutamic pyruvic transaminase and significant effect on absolute heart weight was observed at 7 weeks, depressed serum alkaline phosphatase was observed at 13 weeks and elevated glucose at 7 and 13-weeks. At 7 weeks, the lungs of necropsied animals showed scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single exposed male rat. A LOAEC of 4.2 mg/m^3 was identified based on the blood effects in male rats.

Mechanistic studies

Mechanistic studies on the pulmonary effects of anionic surfactants have been studied in dogs, rabbits, and sheep exposed to DOSS.

Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to DOSS in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) [5, 6]. Anesthetized dogs were exposed *via* a ventilator to particle sizes of

0.5 to 15 μm with an MMAD of 3 μm (no GSD reported). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Alveolar-capillary barrier permeability studies using radiolabeled aerosol tracers have evaluated whether detergents effect the surfactant layer to increase alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary elimination of radiolabeled diethylenetriamine pentaacetic acid (DTPA; CASRN 67-43-6) a relatively small hydrophilic molecule, indicating an increased alveolar permeability after detergent exposure [64-69]. In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occurs with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in rabbits exposed to multiple dilutions (0.125, 0.25, 0.5, and 2%) with a MMAD of 1.7 μm of the

liquid detergent [67]. Studies also evaluated the elimination of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA [66, 70]. Wang *et al.* (1993) [6] observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which was attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies was hypothesized to be a result of increased alveolar surface tension, which may result in increased permeability by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores [6, 64]. However, as noted, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation [1].

Cationic Surfactants

In vivo studies

Three acute inhalation toxicity studies were identified for cationic surfactants; one study each for DDAC, dioctadecyldimethylammonium chloride (DODMAC; CASRN 107-64-2), and BAC. DDAC, which is corrosive to the skin and severely damaging to the eye [71],

was tested in rats (5/sex/dose, unspecified strain) exposed *via* inhalation to 0.05, 0.09, 0.13, 0.25, 1.36, or 4.54 mg/L (50, 90, 130, 250, 1,360, or 4,540 mg/m^3) for 2 hours with an observation period of 14 days (no additional exposure conditions reported). An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs [72]. A similar quaternary amine, DODMAC, which is irritating to the skin and causes serious damage to the eyes [73], was tested in albino rats (10 males, strain not specified) to the test substance (1:29 distilled water) *via* inhalation at 180 mg/L (180,000 mg/m^3) for one hour and observed for 14 days (no additional exposure conditions reported). No mortalities were reported and observed treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose, and labored respiration. All animals appeared normal one day after dosing. The LC_{50} (1 h) was > 180 mg/L. BAC, which is corrosive to the skin and causes severe eye damage [74, 75], was tested in female Wistar rats (5/group) exposed *via* nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (MMAD and GSD not

reported) [75]. The LC_{50} was reported to be approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as tumor necrosis factor (TNF)-a, interleukin (IL)-6. Indicators of respiratory tract damage, including increased LDH, total protein, and lung weight were also observed.

Three repeated dose inhalation studies of three different exposure durations were identified for DDAC: 14-day, 28-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed *via* whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 for 6 hours/day, 7 days/week [76]. The study authors reported an MMAD of 1.86 µm and a GSD of 2.75; however, individual values for each exposure concentration were not provided. Mild effects were noted in cell differential counts and cell damage parameters in BALF, in addition to inflammatory cell infiltration, and interstitial pneumonia at the medium and high exposures. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure (4-week) study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed *via* dynamic nose-only inhalation to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 DDAC (MMAD 1.4, 1.5, and 1.9 µm, GSD 1.83, 1.86, and 1.87, density not reported) for 6 hours/day, 5 days/week [10]. Body weights were significantly reduced in the high exposure group (males only) on days 14, 21, and 25. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. BALF analysis indicated that, at the high concentration, neutrophils and eosinophils increased with a concomitant decrease in macrophages. Histopathological findings in the nasal cavity were graded according to severity from minimal to severe and increased mucus of the respiratory epithelium in males and females was minimal to moderate at all exposures and mild to moderate ulceration of the nasal cavity in males and females in the high concentration group only. In males, there was an increase in cell count and total protein across all exposures. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. A conservative LOAEC of 0.08 mg/m^3 was previously identified by the Agency based on increased

mucus of the respiratory epithelium and increased LDH; however, due to the mild effects and low number of animals/group, the effects were not statistically significant [10].

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole-body exposure chambers for 6 hours/day, 5 days/week [77]. The MMAD of the DDAC aerosol was 0.63 µm, 0.81 µm, and 1.65 µm, and the geometric standard deviations were 1.62, 1.65, and 1.65 in the low (0.11 ± 0.06 mg/m^3), the middle (0.36 ± 0.20 mg/m^3) and the high (1.41 ± 0.71 mg/m^3) exposure groups, respectively. Body weight influenced by exposure to DDAC with the mean body weight approximately 35% lower in the high exposure (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high exposure (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and LDH were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups and in males in the high concentration group only, while inflammatory cell infiltration and interstitial pneumonia was observed in both the mid- and high-concentration groups. Tidal volume and minute

volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations of 0.8, 4 and 20 mg/m^3 for 6 hours/day, 7 days/week [78]. Mean concentration of BAC in the whole-body exposure chambers of the T1 (0.8 mg/m^3), T2 (4 mg/m^3) and T3 (20 mg/m^3) groups during the exposure period was 0.84 ± 0.09, 4.01 ± 0.12, and 19.57 ± 0.97 mg/m^3, respectively; the MMAD of the aerosols was 1.614, 1.090, and 1.215 µm, respectively, and the GSD was 2.00, 1.86, and 1.51, respectively. The MMAD and GSD were confirmed to be within the range recommended by the OECD (2018) [79]. Among the general signs observed during the exposure period, soiled perineal region, rales, and discharge were continuously observed during the 2-week recovery period. Rales and deep respiration were observed in the high concentration. Exposure-related effects in the upper airway included nasal discharge at

the low and mid concentrations, and. ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high concentrations.

In the lower airways, degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens were observed in the mid and high concentration male groups and high concentration dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchioles were observed in both males and females. Effects indicating tissue injury included squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole. In the BALF analysis, the concentration of reactive oxygen species (ROS)/reactive nitrogen species (RNS), IL-1β, IL-6, and macrophage inflammatory protein (MIP)-2 decreased concentration-dependently at the end of the exposure period, which indicated oxidative damage, but

did not show a concentration-dependent change at 4 weeks of recovery. The concentrations of TNF-α, IL-4, and transforming growth factor (TGF)-β did not show changes associated with test substance exposure. Relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors identified a LOAEC of 0.8 mg/m^3 based on effects in the nasal cavity.

Mechanistic studies

In vitro assays have demonstrated that cytotoxic effects of cationic surfactants have significantly greater toxicity to non-polarized than polarized mammalian cells [80]. In this study, cell viability as measured by LDH and MTT assays in non-polarized HeLa immortal cell line cells and fetal skin dendritic cells (FSDC) was more sensitive to the effects of different cationic surfactants of varying alkyl chain length and polar head groups than polarized cell lines Madin-Darby Canine Kidney (MDCK) and Caco-2. The cationic surfactant toxicity was shown to occur well below their CMC, and greater toxicity was observed with alkyl lengths of 10-12 than 14-16; however, this association

was not strictly a linear relationship. In addition, the cationic surfactants with a larger polar head group (*i.e.*, benzalkonium) were 2-5 times more toxic than cationic surfactants with a more localized charge (*i.e.*, trimethylammonium).

The effects of BAC on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells has been replicated *in vitro* using a dynamic culture condition that reflects the natural microenvironment of the lung to simulate the contraction and expansion of breathing [81]. Normal breathing levels were simulated (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of exposure rate during the cell culture. The system assessed toxicity by culturing submerged cells with different BAC concentrations (0, 2, 5, 10, 20, and 40 μg/mL) under static and dynamic culture conditions. Following a 24-hr exposure to BAC, cellular metabolic activity, IL-8, and ROS levels were significantly affected, compared to untreated cells, when using either static or dynamic cell growth conditions. The dynamic culture system,

which more closely mimics lung conditions, showed a higher toxic response to BAC as indicated by increased ROS levels.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The animal inhalation toxicity data identified by the literature search and PODs from the studies are summarized in Table 3. It should be emphasized that new information (*e.g.*, study data, POD derivation approaches, mechanistic information, *etc.*) may lead to updates/additions to this table. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human equivalent inhalation exposure [20]. The exposure duration adjustment and DAF approaches were described above. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table 3 for each of the toxicity studies from which PODs could be identified. However, other approaches to dosimetry adjustment may be considered relevant (*e.g.*, use of the multiple-path particle dosimetry model [MPPD]).

For the nonionic surfactant, octylphenoxypolyethoxyethanol, the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with effects in the thoracic region; therefore, the RDDR of 0.812 was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, the extrathoracic RDDR (0.0.111) was used to calculate the HEC. In the 28-day inhalation study with DDAC, effects were observed throughout the respiratory tract, including the nasal cavity; therefore, the thoracic RDDR (0.854) was used for calculating the HEC. Similarly, for the cationic surfactant, BAC histopathological cellular changes were observed in the nasal cavity and lungs, indicating the extrathoracic RDDR (0.106) should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table 3.

Table 3. Inhalation Toxicity Points of Departure and Human Equivalent Concentrations (HEC) for Surfactants.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/Type	Study POD	Value (mg/m^3)	Reference	Density (g/cm^3) at 20 °C[1]	RDDR Model Input Parameters		RDDR[2]	HEC (mg/m^3)
							MMAD (μm)	GSD		
Nonionic	octylphenoxypolyethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body	LOAEC	5.3	[8]	0.998 water vehicle	1.80	1.80	$RDDR_{ET}$ = 0.196 $RDDR_{TB}$ = 1.367 $RDDR_{PU}$ = 0.564 **$RDDR_{TH}$ = 0.812** $RDDR_{TOT}$ = 1.547	1.0 7.2 3.0 **4.4** 8.2
Anionic	oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD TG 412)	LOAEC	< 6	[62]	0.7893 ethanol vehicle	1.16	2.12	**$RDDR_{ET}$ = 0.111** $RDDR_{TB}$ = 2.008 $RDDR_{PU}$ = 0.447 $RDDR_{TH}$ = 0.742 $RDDR_{TOT}$ = 0.970	**< 0.6** < 12.0 < 2.7 < 4.5 < 5.8
Cationic	DDAC	4-week, 6 hr/d, 5 d/wk; nose-only	LOAEC[3] (lung effects)	0.08	[10]	NR	1.60	1.85	$RDDR_{ET}$ = 0.211 $RDDR_{TB}$ = 1.674 $RDDR_{PU}$ = 0.539 **$RDDR_{TH}$ = 0.854** $RDDR_{TOT}$ = 1.607	0.02 0.13 0.04 **0.07** 0.13
	BAC	14-day, 6 hr/d, 7 d/wk; whole body	LOAEC (nasal effects)	0.8	[78]	0.998 water vehicle 2% dose solution	1.31	1.79	**$RDDR_{ET}$ = 0.106** $RDDR_{TB}$ = 1.988 $RDDR_{PU}$ = 0.528 $RDDR_{TH}$ = 0.815 $RDDR_{TOT}$ = 0.991	**0.08** 1.59 0.42 0.65 0.79

MMAD: Mass Median Aerodynamic Diameter of inhalation study aerosol, average values listed; GSD: Geometric Standard Deviation of the inhalation study aerosol, average values listed; RDDR: Regional Deposited Dose Ration; ET: Extrathoracic; TB: Tracheobronchial; PU: Pulmonary; TH: Thoracic = TB + PU; TOT = ET + TB + PU.

[1]Exact density of administered compounds not reported (NR); vehicle density was listed when provided.

[2]RDDR values are for male and female animals, whichever was lower, as calculated using RDDR.exe and described in the Supporting Information file at "Section 2 RDDR Modeling".

[3]conservative estimate: effects were not statistically significant.

NA: Data not available or RDDR values could not be calculated from the available information.

Benchmark Margin of Exposure Analysis

The substances shown in Table 3 provide representative examples of PODs that may be applied to new chemistries that meet the Surfactant Criteria, after evaluating whether the chemical substances in Table 3 are appropriate toxicological analogues for read-across to the new chemical substance. Alternatively, the notifier may propose a different representative POD and/or analogue, if supported by scientific evidence. If a determination cannot be made on whether one of these chemical substances (Table 3 or other representative analogue) is an appropriate toxicological analogue, then the relevant substance from Table 3 should be identified as a comparator substance[4] for use in the Tiered-Testing Strategy, discussed below. Though the initial starting point for

[4] A comparator substance is one that may possess similar properties to the new chemical substance and for which inhalation toxicity data are available. EPA may "read-across" the toxicity data from the comparator substance to the new chemical substance when no other information is available. The tiered-testing approach for this category is designed to determine whether this practice may be refined or supported by additional data. As such, the comparator substance should be used in side-by-side testing in Tiers I-III with a new chemical substance to aid with interpreting the test results of the new chemical substance.

deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs or consideration of the representativeness and comprehensiveness of the available database to characterize potential effects after inhalation exposure. As shown in Table 3, the uncertainty factors were based on RDDRs that were used as DAFs to account for animal-to-human toxicokinetic differences.

In the case of surface-active substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (See, *e.g.*, EPA, 2020 [82]). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a direct-acting adverse outcome pathway (AOP) such as the one regarding surfactant that is under

development [83], the default values for UF_H and UF_A are each reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1. In order to apply these reductions, the following criteria must be established:

1. A description of the AOP,
2. A discussion of why the AOP is unlikely to differ between humans, in the case of UF_H, or between animals in comparison to humans, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is addressed by the use of dosimetry modeling.

When the above criteria are met, application of the appropriate DAF (*e.g.*, the RDDR for particles) should still be applied, given that deposition is the most appropriate dose metric for assessing acute/subacute effects from surface-active agents. However, since the DAF accounts for the toxicokinetic component of UF_A, the remaining value of 3 (*i.e.*, $10^{0.5}$ or 3.16) should be retained for the toxicodynamics component of the UF_A.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all three of the above criteria, then a value of 3 may be applied. The reduced value represents a reduction in the TK component of this UF to 1, with the remaining value of 3 accounting for the TD component.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of dosimetric adjustments for quantifying an HEC or when the available information does not support each of the above three criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied, which represents a reduction in the TK component to 1 and application of a value of 3 for the TD component.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL with an appropriate biologically significant benchmark response (*e.g.*, 10% extra risk for quantal data or 1 standard deviation for continuous data) should be calculated and a value of 1 should be applied for this area of uncertainty.

The above considerations and approaches support the application of a benchmark MOE ranging from 10 (*i.e.*, $10^{0.5} \times 10^{0.5} \approx 10$) to 1,000 depending on the chemical substance identified as an appropriate toxicological analogue and available data on the new chemical substance. In those instances where the data are too limited to determine when one of the chemical substances is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined includes a number of uncertainties and limitations, including those associated with extrapolating the hazards identified from the chemical substances shown in Table 3. Uncertainties associated with using animals to estimate human toxicity are recognized and methods are presented to reduce extrapolation uncertainties [84]. Procedures for the adjustment of exposure durations for inhalation exposures and application of DAFs to derive HECs are well-established procedures for reducing uncertainties associated with the TK aspects of animal-to-human extrapolation factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors) [19, 20]. Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of the chemical substances in Table 3 to new chemical substances needs to be carefully considered,

with attention given to the influence of additional functional groups on the toxicity of the new chemical substance, as well as the particle properties (MMAD, GSD, and density) of the candidate new chemical substance. Simulation studies using dosimetry models such as the RDDR or multiple-path particle dosimetry (MPPD) models can inform these considerations. Additionally, the risk assessors should consider if a different comparator substance and/or POD may be more appropriate (*e.g.*, based on new scientific information of the new chemical substance profile). Risk assessors should consider the surface tension and CMC criteria (Table 2) compared to these measurements for the new chemical substance and the influence of the presence or absence of additional functional groups on these criteria (*e.g.*, would a particular functional group increase or decrease toxicity, for example by another mechanism of action). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, the hazard(s) and risk(s) should be characterized using the chemical substance as a toxicological analogue to the new chemical substance. Of course, uncertainties regarding this extrapolation should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that a chemical substances (Table 3 or other relevant analogue) is comparable to or represents an acceptable toxicological analogue to the new chemical substance, then the Tiered-Testing Strategy provided could be used to determine whether the new chemical substance has lower, comparable, or higher toxicity to the relevant chemical substance in Table 3, as a comparator substance and not as a toxicological analogue. Prior to conducting such testing, the scientific basis for selecting the comparator substance to the new chemical substance should be understood and a rationale provided as to why the comparator substance will be used for testing.

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" [85]. Moreover, the

amended TSCA requires entities undertaking voluntary testing for submission to EPA to first "…attempt to develop the information by means of an alternative test method or strategy …before conducting new vertebrate testing…" [85]. Additionally, in 2019, EPA was directed to prioritize efforts to use NAMs to reduce animal testing [86]. Multiple NAMs exist which can be used to assess hazards and risks of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing and *in vitro* methods to specifically assess respiratory toxicity. Several methods are described within a tiered-testing strategy recognizing that these assays are provided as examples and the development of NAMs is advancing rapidly. As such, the NAMs included here should not be considered all-inclusive or a final compilation. EPA strongly encourages the development and use of NAMs, particularly to reduce or replace the use of animals and is open to considering and discussing additional NAMs with PMN submitters during a pre-notice consultation [87].

In the interest of reducing or replacing vertebrate testing and designing a scientifically robust testing approach, when a surfactant is determined to be respirable, EPA encourages evaluating its

potential to cause respiratory tract toxicity using an AOP approach. The OECD provides "An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect" and that "AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning" [88]. AOPs in various stages of development are useful for different purposes and an AOP may be useful even if it has not been formally evaluated by the OECD.

An AOP can be used to help design a testing strategy and to identify NAMs that can query the key events leading up to the adverse outcome. As an example, using the respiratory contact irritant chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CASRN 1897-45-6), Syngenta Crop Protection applied a NAM for the assessment of inhalation toxicology based on an AOP approach [89, 90]. The approach involved derivation of the POD from an *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Similar approaches can be used for surfactants where *in vitro/ex vivo* systems may be used to investigate specific key events in an AOP and confirm that a new chemical substance fits within the boundaries of the Surfactant Category, and therefore, may act like a surfactant (group assignment *via* similar AOP) and/or if other substance-specific properties lead to a predominant type of key event within the AOP. Further, *in vitro* tests may deliver information while avoiding *in vivo* testing or, if considered, provide helpful information on dose-selection for *in vivo* testing.

An AOP connects a molecular initiating event (MIE) to key events, at the cellular, tissue, and organ levels, which lead to an adverse outcome at the organism or population level [91, 92]. For surfactants, proposed MIEs include interaction of the substance with the epithelial lining fluid or lung-surfactant, or the molecular interaction of the substance itself with cell membranes of the epithelium in the respiratory tract. The resulting key events include disruption of airway epithelial cells (AEC) due to loss of lung cell surfactant function and/or the loss of membrane integrity (cellular level key events). These cellular events may lead to different tissue or organ level events (*e.g.*, cytotoxicity and perturbation of AEC, increased alveolar surface tension and alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (*i.e.*, the adverse outcome) (*e.g.*, pneumonia, limited lung function by chronic obstruction (COPD), interstitial fibrosis, *etc.*).

Some *in vitro* tests, such as by capillary surfactometer, may be useful in screening chemicals to be tested for the Surfactant Category, but do not by themselves constitute

adequate tests for acute respiratory tract effects of these chemicals. This information should be taken into consideration within an integrated approach. These assays can be used as part of a weight of evidence evaluation to determine whether to consider animal testing or if a POD can be determined for risk assessment purposes without the use of animals. Each test can provide insight on one key event of the AOP, which collectively, may provide a comprehensive picture of the likelihood of toxicity.

A number of different types of *in vitro* test methods, summarized in Table 4, may be used to query key events in AOPs relevant to the disruption of lung function by surfactants [83]. Clippinger *et al.* (2018) [93] have also described a decision tree and potential key events that can be used to design pathway-based approaches for *in vitro* testing of inhalation exposures.

Table 4. Potential Methods for Evaluating Chemicals in the Surfactant Category.

Level of Biological Organization	*Key Events*	*In Vitro Assay*	*Test System*
Molecular Initiating Events (MIEs)	Interaction with pulmonary surfactant	*In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant interaction, *e.g.*, as described by Sorli *et al.* (2018) [94]
	Interaction with cell membrane and cell membrane components and interaction	Hemoglobin Denaturation Assay, Liposome Assay, and *In Vitro/Ex Vivo* Irritation Assays	• Hemoglobin denaturation assay, *e.g.*, as described by Hayashi *et al.* (1994) [95] • Liposome assay, *e.g.*, as described by Kapoor *et al.* (2009) [96] • *In vitro/ex vivo* eye irritation tests for penetrance, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD TG 492) [97], Bovine Corneal Opacity and Permeability Test (OECD TG 437) [98], Isolated Chicken Eye Test (OECD TG 438) [99], *etc.*
Cellular Level Events	Loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• *In vitro/ex vivo* eye irritation tests for cytotoxicity, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD TG 492) [97], Bovine Corneal Opacity and Permeability Test (OECD TG 437) [98], Isolated Chicken Eye Test (OECD TG 438) [99], *etc.*
			• Cell membrane integrity test (LDH-cytotoxicity assay), cell viability assays (*e.g.*, MTT, resazurin [100], and ATP), TEER [100], or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCVAM, 2006) [101]
Tissue or Organ Level Events	Tissue level events	Human organotypic Airway Cultures	• 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (*e.g.*, EpiAirway™, MucilAir™, SmallAir™, EpiAlveolar™, *etc.*) using the cell membrane integrity and viability assays described under cellular level events [93]
	Tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test, *e.g.*, as described by Hess *et al.* (2016) [102] and Neuhaus *et al.* (2017, 2018) [103, 104]

MIEs

There may be multiple AOPs that would be relevant to the Surfactant Category. The MIE for a proposed AOP under development is the interaction of a substance with lung surfactant, which may lower the surface tension and disrupt lung surfactant function [83]. Sorli *et al.* (2017) [94] developed an *in vitro* lung surfactant interaction assay that specifically measures whether a substance alters the surface tension of pulmonary surfactant. The assay was initially developed for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle that may be relevant for some types of surfactants.

The proposed MIE for another AOP relevant to surfactants is direct interaction with AEC or pulmonary cell membranes, which may be followed by cytotoxicity. While the hemoglobin denaturation and liposome assays and *in vitro* eye irritation assays do not

directly measure effects on membranes of AEC, these assays have been shown to be useful screening approaches for determining the ability of surfactants to interact with cellular membrane components and cell membrane penetration. For example, Hayashi *et al.* (1995) [105] showed that charged surfactant molecules can interfere with charged side chains of the hemoglobin protein. These interactions led to disruption of the three-dimensional (3D) structure of hemoglobin, causing a change in light absorbance that can be measured. Increasing concentrations of SDS and sodium lauroylmethyltaurate (LMT; CASRN 4337-75-1) were tested in this assay and showed concentration dependent increases in hemoglobin denaturation, which correlated with irritation effects in the Draize eye test [95, 105, 106].

The liposome assay can be used to assess disruption of the lipid bilayer of the membrane from interaction with surfactant chemistries. Kapoor *et al.* (2009) [96] measured the release of calcein dye from liposomes following exposure to various surfactants and showed a positive correlation with these findings and data from the Draize eye test. The hemoglobin denaturation and liposomal assays were both optimized and validated against eye irritation data; therefore, these assays may provide

an opportunity to evaluate the effects of surfactants on the respiratory tract. Further *in vitro* testing of known surfactants with existing data alongside new chemical substances will help benchmark the results. Nonetheless, these assays are useful for understanding the potential toxicity of a new surfactant substance to AEC or pulmonary cell membranes.

The use of *ex vivo* eye irritation studies may provide indirect measures of surfactants on cell membranes, which may be relevant to the effects observed from comparator substances in the respiratory tract. For example, Bader *et al.* (2013) [107] reported that the Bovine Corneal Opacity and Permeability (BCOP) assay was effective at demonstrating that nonionic (*i.e.*, octylphenoxypolyethoxyethanol), anionic (*i.e.*, SDS), and cationic (*i.e.*, BAC) substances cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. The permeability score was more predictive of eye irritation than the ocular opacity score for octylphenoxypolyethoxyethanol and SDS, whereas with BAC, the opacity score was more predictive of eye irritation than the permeability score.

Therefore, a systematic investigation of opacity and permeability measures of surfactants tested in the BCOP may be helpful with elucidating toxicity to AEC or pulmonary cell membranes.

In addition, information on the potential of a substance to cause skin irritation (*e.g.*, OECD TG 439 [108]) and/or skin corrosion (*e.g.*, OECD TG 431 [109]) *in vitro*, can provide supporting evidence of the potential for a substance to cause similar irritant or corrosive effects in respiratory tract cells. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, demonstrated by DDAC that causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 [110].

Cellular Level Effects

In vitro/ex vivo assays can be used to assess key events on the cellular level in AOPs relevant to the Surfactant Category (see Supplemental Table 1 in Clippinger *et al.*, 2018 [93]). For general cytotoxicity (Table 4), cell lines are available that are known to be

sensitive to the effects of surfactants. Use of the BALB/c 3T3 NRU cytotoxicity test to reduce animal testing by estimating starting doses for acute oral toxicity testing has been reviewed and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and is an OECD guidance document [101, 111]. The surfactants with known inhalation toxicity (*e.g.,* octylphenoxypolyethoxyethanol, oleoyl sarcosine, DDAC, or BAC) should be tested in parallel with the new chemical substance to benchmark the results, thereby providing reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

Tissue or Organ Level Effects

Based on the results of testing cellular level key events, it may be necessary to perform additional testing. Human and animal airway epithelia are composed of multiple cell types that each have specialized functions, making the use of 3D co-culture assays more physiologically relevant than 2D monoculture systems. Thus, several human organotypic airway models have been developed that allow for the assessment of

multiple endpoints in 3D culture systems. Two commonly employed systems are EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively.

Organotypic airway cultures, such as EpiAirway™ and MucilAir™, [112], take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain homeostasis for months in culture. Because of these characteristics, these human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Dosimetry models such as the RDDR or MPPD can be used to predict the anatomical area and internal amounts delivered in various regions of the respiratory system for humans under the target inhalation exposure scenario for the given use case. Different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. MucilAir™ provides a 3D co-culture model of cells from nasal, tracheal

or bronchial sites, and SmallAir™ provides a co-culture model of cells from small airways. EpiAirway™ is composed of a co-culture of normal human tracheal/bronchial epithelial cells, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts (available with and without macrophages).

Exposure of respiratory tract 3D co-culture models to aerosols at the air liquid interface (ALI) using an *in vitro* exposure system, such as those available from Vitrocell® Systems, provides an exposure more comparable to real-life scenarios for inhaled aerosols. The tradeoff has been a lower throughput compared to *in vitro* two-dimensional exposure systems; however, 3D tissue models and ALI exposure systems are now available in a 96-well format. Dilution in medium and interaction with medium components does not occur in the ALI exposure systems as in submerged culture systems. The respiratory tract 3D co-culture models are more physiologically relevant because there is an interaction of the aerosol with a mucus or surfactant layer, as in humans.

Exposures of these organotypic cultures at the ALI can be combined with other assays for assessing cell function and viability in an AOP approach. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays (such as MTT, resazurin, or ATP assays), have all been reported for use with these cultures. Further, multiple assays can be performed on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant octylphenoxypolyethoxyethanol at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, provided dosimetry models are available for translation of the internal dose achieved under culture conditions to an equivalent inhalation exposure for the human scenario of interest. Examples of *in vitro* dosimetry models to predict particle doses for submerged cell culture include the *In vitro* Sedimentation, Diffusion and Dosimetry model (ISDD) [113] and the *In vitro* Sedimentation, Diffusion and Dissolution Dosimetry (ISD3) model [114].

Significant progress has been made toward achieving the objectives to use high-throughput *in vitro* assays and computational models to evaluate potential adverse effects of chemical exposures [16, 115]. To translate the effects to higher levels of biological organization, a battery of assays with varying complexity and physiological relevance may be needed. The 3D human organotypic airway cultures add evidence to an AOP approach and increase confidence in the physiological relevance to humans.

Precision-cut lung slices (PCLS) provide an additional method to develop key event data using *ex vivo* cultures of human or rodent lung slices. The PCLS can be used to measure multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure [103, 104, 116]. PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often used in toxicological and anatomical studies

regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema [117]. Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be evaluated. One further advantage of PCLS is that the assay can be performed on multiple species to determine inter-species variability in susceptibility.

Human PCLS, derived from, for example, rejected but otherwise healthy transplant tissue, can be used to measure cell/tissue viability, local respiratory inflammation, and physiological function. These endpoints can be measured in single and repeated exposures in a metabolically competent system within the normal architecture of the lung in a more relevant model system, replacing the need for animal testing [103, 104, 116].

When human PCLS are not available, rat PCLS provide an alternate option. The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed correlation with *in vivo* LC_{50} values [102]. The use of rat PCLS reduce the

number of animals used to conduct dose response studies, as compared to *in vivo* inhalation tests. From a rat lung (1 g), approximately 200 slices can be prepared. In general, for each test substance concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested using tissue from a single rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures *via* liquid media or, with additional adaptations, air. As such, rodent PCLS meet the goal of reducing animal testing, although dosimetry models for their translation to HEC are not yet developed. Mechanistic rodent and human PCLS studies may be conducted in parallel to understand species specific difference in toxicological effects. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations of an AOP Approach to the Surfactant Category

A number of *in vitro* assays have been discussed as to their potential utility for assessing key events in an AOP(s) relevant to characterize the Surfactant Category. Uncertainties and limitations associated with these assays are discussed for each of the

above testing systems, as well as others [93]. It is important to consider that these assays were not systematically tested using surfactants. Nonetheless, these assays can be conducted using an AOP approach to provide information on whether a new chemical meets the Surfactant Category criteria and/or to understand whether the new chemical may be more or less bioactive or toxic than the sub-category comparator chemicals. EPA will generally use the framework and analogue toxicity data identified in this investigation to assess potential risks from surfactants.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment continues to evolve. A fit-for-purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, and domain of applicability for evaluating a new method to inform specific decisions has emerged from the regulatory science community to address the challenges posed for validation of NAMs [16, 118-120]. Such fit-for-purpose validation approaches are intended to be flexible and adaptable and to provide data sets, prediction analysis results, inference models, *etc.* in a transparent manner that enable

other scientists to confirm the performance of the assays and inference models, as well as evaluate the rationale for using these assays in a specific decision context.

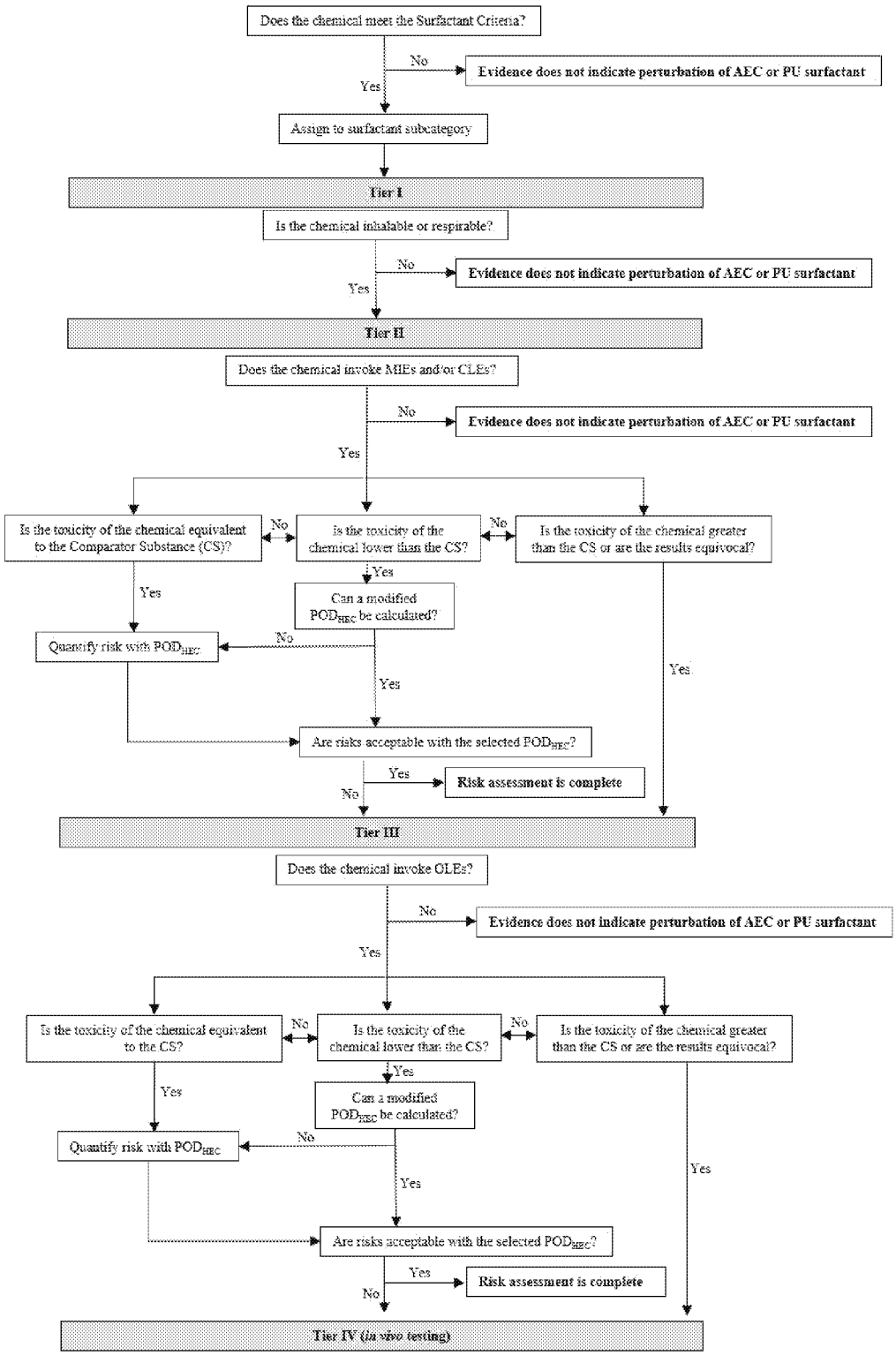
Once such fit-for-purpose scientific evaluations are documented, there are several ways that these assays can be used to reduce and replace animal testing. First, testing can be performed based on an AOP approach to evaluate the potency of new surfactants versus a comparator substance within the relevant subcategory that has repeated exposure inhalation toxicity data. Second, depositional data using models such as the RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Tiered-testing Strategy

The first step in the tiered-testing strategy is to determine if the evaluated substance meets the Surfactant Criteria. If so, then assign the substance to the appropriate

surfactant subcategory (nonionic, anionic, or cationic) and determine whether any of the representative subcategory chemicals may serve as an acceptable toxicological analogue for risk assessment or as a comparator substance for tiered testing. If a representative subcategory chemical is determined to be an acceptable toxicological analogue to the new chemical substance, then quantify risks using the toxicological analogue. If the MOE is equal to or greater than the benchmark MOE, then tiered testing is not required on the new chemical substance. If the MOE is lower than the benchmark MOE or if a determination cannot be made on whether any of the representative subcategory chemicals are acceptable toxicological analogues, then proceed with tiered testing using the most appropriate subcategory chemical as a comparator substance to the new chemical substance. As detailed below, the tiered-testing strategy commences with the least complex, most efficient testing methods, and at each subsequent tier, the complexity of the test system increases, commensurate with key events in proposed AOPs relevant to the Surfactant Category, to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system. It is envisioned that both the new chemical substance and the comparator substance will

be evaluated side-by-side in the NAM assays. The results of these studies may lead to the conclusion that the comparator substance is an acceptable toxicological analogue to the new chemical substance. Alternatively, the results may support that higher tiered testing is warranted, particularly when the new chemical substance has higher toxicity than the comparator substance. If *in vivo* testing is conducted, it may not be necessary to run the comparator substance in the *in vivo* tests, given that suitable inhalation studies are available on the comparator substances. A summary of the proposed tiered-testing strategy is provided in Scheme 1 and discussed further below.



Scheme 1. Proposed tiered-testing strategy for general surfactants.

Tier I—Physicochemical properties

Surfactants are proposed to cause a specific sequence of biological events in the respiratory tract if they are inhaled. Manufacture, processing, or use of a surfactant in an inhalable form, (*i.e.*, ≤ 100 µm aerodynamic diameter) is therefore, an initial consideration of the potential for a surfactant to cause toxicity to the respiratory tract. Particle size is an established parameter for determining inhalability/respirability of particles/droplets. Several validated test methods exist for determining potential inhalability/respirability, *i.e.*, particle size, of a new chemical substance (*e.g.*, OECD GD 39 [79], ISO 21501-1:2009 [121], OECD TG 110 [122], and OPPTS 830.7520 [123]). The studies shown in Table 3 suggest that the total respiratory tract may be affected from surfactants; therefore, inhalable forms (≤ 100 µm) were identified as the most relevant for quantitative inhalation risk assessment. As a practical matter, a particle size cutoff of greater than 1% inhalable particles/droplets by weight (wt%), determined in a well conducted study using a valid measurement method will generally be considered as triggering a quantitative assessment of inhalation toxicity on a new chemical

substance meeting the Surfactant Criteria. EPA will generally assess the potential inhalation toxicity for a new surfactant chemical substance when the manufacture, processing or use results in greater than 1% (by weight) of the surfactant particles/droplets having a particle size of less than 100 μm. This wt% cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials [124].

If inhalable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information to determine whether a new chemical substance invokes MIEs and cellular level key events. In order to determine the best approach for *in vitro/ex vivo* testing, a pre-notice consultation with EPA is highly encouraged. In general, the testing approach in this tier

should include a combination of assays, such as one that measures epithelial lining fluid/cell perturbation or pulmonary surfactant interaction/loss of function, one that measures cell membrane interaction/disruption/penetration), and one that measures loss of barrier integrity or general cytotoxicity (see Table 4). *In vitro*/*ex vivo* eye irritation studies may also be used to demonstrate cell interaction or penetration and general cytotoxicity, and *in vitro* skin irritation/corrosion studies can provide supporting evidence of possible irritant or corrosive effects in the respiratory tract.

For each assay, the comparator substance for the respective subcategory of surfactants should be tested under identical conditions. Further, the particle size distribution data may be used with dosimetry models such as RDDR or MPPD to aid with identifying the regions in the respiratory tract where deposition is expected to occur and the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc.*

Notwithstanding the uncertainties with the above assays, each may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE) for the purpose of evaluating the relative potency of the new chemical substance versus the comparator substance. Several investigations have provided insight on approaches for accomplishing this, although with different assay systems [89, 90, 125]. In doing so, a weight of scientific evidence evaluation should be performed considering the structural features, physicochemical properties, and assay results on the new chemical substance versus the comparator substance. Based on this evaluation, the most biologically relevant endpoint(s) should be used to calculate a POD. BMD modeling may be applied to derive a $BMCL_{1SD}$ metric, as a possible metric, although the metric of one standard deviation should be used with caution [126]. Alternative metrics should be considered, as our understanding evolves for various *in vitro* assays and endpoints. For example, the pharmaceutical industry has used fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc.*) [127]. Regardless of the metric used, a justification for its selection should be provided. In those situations where data are not amenable to BMD modeling,

the *in vitro* concentration tested should be determined based on the expected HEC for the appropriate subcategory (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC.

Given that the understanding of IVIVE is evolving, assay results should be interpreted in a manner consistent with the weight of scientific evidence, as noted above, while recognizing that uncertainties are often dealt with by erring on the side of conservativism. Therefore, the following initial default criteria are proposed for utilizing the assay results, and when possible, the IVIVE estimates. These criteria are consistent with EPA's approach for evaluating non-animal skin sensitization data [128], while recognizing that the weight of scientific evidence may support an alternative interpretation to the default criteria.

The Tier II assays evaluate biologically relevant endpoints representing key events in AOPs relevant to the Surfactant Category. The results of the comparator substance and the new chemical substance in these assays provide a basis for evaluating the

suitability of using the comparator substance to evaluate toxicity of the new chemical substance. Consideration should also be given to differences in the specific physicochemical properties influencing inhaled deposition (*i.e.*, MMAD, GSD, and density) between the comparator substance the new chemical. Dosimetry models such as RDDR and MPPD can be used to inform these comparisons.

If comparable toxicity is observed between the comparator substance and the new chemical substance in the Tier II assays, the POD_{HEC} from the comparator substance may be appropriately used as a toxicological analogue for quantifying the MOE. If calculated risk is acceptable stop at Tier II, otherwise proceed to Tier III.

If lower toxicity is observed for the new chemical substance versus the comparator substance in the Tier II assays, then these data should be used to determine if a modified POD_{HEC} can be quantified for the new chemical substance. If this is possible, the modified POD_{HEC} for the new chemical substance should be used for quantifying the MOE. If calculated risk is acceptable, then stop at Tier II. However, if it is not possible to

calculate a modified POD_{HEC}, then the comparator substance POD_{HEC} could be used as a worse-case toxicological analogue for risk assessment. If no acceptable risk can be calculated, proceed to Tier III.

If greater toxicity is observed with the new chemical substance versus the comparator substance in the Tier II assays, suggesting risks would be identified as unacceptable, proceed to Tier III. Alternatively, there may be scientifically justified reasons for an alternative interpretation, which should be clearly articulated with the weight of scientific evidence evaluation. Otherwise, it may be necessary to proceed to Tier III.

If the results from the Tier II assays are equivocal (*i.e.*, they do not demonstrate comparable or lower toxicity of the new chemical substance versus the comparator substance), and there is no clear rationale or explanation, then proceed to Tier III testing because the data are too uncertain to make a reasoned evaluation on the potential health risks, following potential inhalation exposures.

Tier III – 3D Human Airway Models/PCLS Assay

Several testing options are available for evaluating tissue and organ level key events in an AOP relevant to the Surfactant Category. The test system employed should focus on evaluating effects in the respiratory tract at the predicted sites of deposition (*e.g.*, ET, TB and/or PU regions), based on the particle size distribution data generated under Tier I and using RDDR or MPPD modeling. A justification for using a system(s) should be provided and may be discussed with EPA as part of a pre-notice consultation. Representative test systems include those listed in Table 4.

Based on the results of the 3D-construct and/or PCLS testing, IVIVE may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II [112]. Further,

application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use or if the Tier III test data are not amenable for developing a POD_{HEC}, then proceed to Tier IV.

Tier IV – *In vivo* studies

Strategic *in vivo* testing may be considered as a last resort to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the comparator substances in a subcategory has representative toxicological properties to the new chemical substance, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving modified POD_{HEC}s. A pre-notice

consultation meeting with EPA is strongly encouraged prior to initiating any vertebrate animal testing. This point is especially important because TSCA section 4(h)(3) indicates that any person developing information for submission under TSCA section 5 on a voluntary basis shall first attempt to develop the information by means of an alternative test method or strategy identified by EPA before conducting new vertebrate animal testing [85].

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with epithelial lining fluid/pulmonary surfactant and/or disrupting cellular membranes and epithelial cytotoxicity. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests should be considered:

- Step 1: OECD TGs 433, 436, and 403 address acute inhalation toxicity testing. OECD TG 433 is based on evident clinical signs of toxicity rather than death as an endpoint (refinement) and TG 436 uses fewer of animals (reduction), and therefore, they should be

considered before TG 403. Any protocol modifications should be discussed with EPA during a pre-notice consultation meeting.**

- Step 2: 5-Day inhalation study with a 14-day observation period** to address progression/resolution of effects. The OECD TG 412 [129] should be used, but the exposure duration should be 5 days.

**Modifications may include pulmonary function testing (if measurable), analysis of BALF, LDH release, complete histopathological analysis of the respiratory tract and blood oxygen (pO_2) content. OECD TG 412 and OECD GD 39 [79] should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition [130].

The results of the *in vivo* testing should be used for reassessing and recharacterizing the risks of the new chemical substance.

CONCLUSIONS

The overall objective of this investigation was to develop a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. This investigation developed physical-chemical properties, *i.e.*, the Surfactant Criteria, assessors and product stewards can use for determining whether a new chemical substance can be considered a surfactant. Further, properties and characteristics are provided to divide the Surfactant Category into sub-categories for nonionic, anionic, and cationic surfactants, which is important from a toxicological perspective. A systematic literature search and review were conducted to identify data to define a Surfactant Category and substances from which PODs were identified from inhalation toxicity studies. To facilitate chemical comparisons, animal toxicity studies that could be used to derive PODs for risk assessments were identified for at least one chemical substance for each sub-category and converted to HECs using established methods developed by EPA. Finally, a tiered-testing strategy for generating *de novo* data for new surfactant substances is provided that integrates a variety of currently

available NAMs using an AOP framework. The use of this tiered-testing strategy will inform the available data on surfactants and provide greater confidence in the use of non-vertebrate testing approaches for assessing the potential risks of new chemical substances. It also offers advantages to regulators, the regulated community, and consumers because: 1) integrating NAMs into a category testing approach supports EPA, TSCA and product stewardship goals of reducing and replacing vertebrate animal testing; 2) decision analysis for higher tiered testing takes into consideration mechanistic responses, dosimetry, and exposure information; and 3) it encourages development of mechanistic data to advance the understanding of the potential inhalation toxicity of surfactants, which will drive the development of newer and safer chemistries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information file contains the following:

Section 1. Systematic Literature Review

Section 2. RDDR Modeling Outputs

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Notes

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SUPPORTING INFORMATION FOR "SURFACTANTS CATEGORY: THE APPLICATION OF A NEW APPROACH METHODOLOGY (NAM) FOR ASSESSING INHALATION RISKS UNDER THE AMENDED TOXIC SUBSTANCES CONTROL ACT"

1. SYSTEMATIC LITERATURE REVIEW

A. Initial Literature Search

i. Search Strategy

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the respiratory tract of exposed humans, investigated respiratory tract outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the respiratory tract of humans and experimental animals. The search query string is presented in [REF _Ref46547342 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, a Supplemental Literature Search performed on April 13, 2018, which included an expanded list of MeSH, query, and text words. Further details are provided under Section 1, Subsection B.

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in [REF _Ref46547473 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. PECO criteria for screening literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (e.g., case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Supplemental Literature Search performed on April 13, 2018. Further details are provided under Section 1, Subsection B.

ii. Additional Search Strategies

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. The resources searched for pertinent gray literature are listed in [REF _Ref46547609 \h * MERGEFORMAT] The chemicals and compound groups identified from the Initial Literature Search and used for gray literature searching are listed in [REF _Ref46547652 \h * MERGEFORMAT]. The screening methods for this search included manual screening of titles/abstracts and the full text reports using the PECO criteria shown in [REF _Ref46547473 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. List of resources searched for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]
EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table [SEQ Table * ARABIC]. List of resources searched for gray literature.

NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table [SEQ Table * ARABIC]. Surfactants, constituent names, and CASRNs used for searching gray literature.

Chemical Group or Constituent Name	**CASRN**
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). A Supplemental Literature Search was performed in April 2018. The details of this search are provided in the section titled "Supplemental Literature Search". The Supplemental Literature Search was used to identify additional studies or data related to lower respiratory tract (LRT) effects of surfactants that became available after the original search was conducted.

iii. Literature Search and Screening Results

The summary results of the literature searches and screening effort are presented graphically in **[REF _Ref46547725 \h * MERGEFORMAT]**. The PubMed search in 2016 identified 43 potentially relevant references for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Supplemental Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded 25 potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full

text review included 35 papers on compounds that were not used as surfactants or did not.

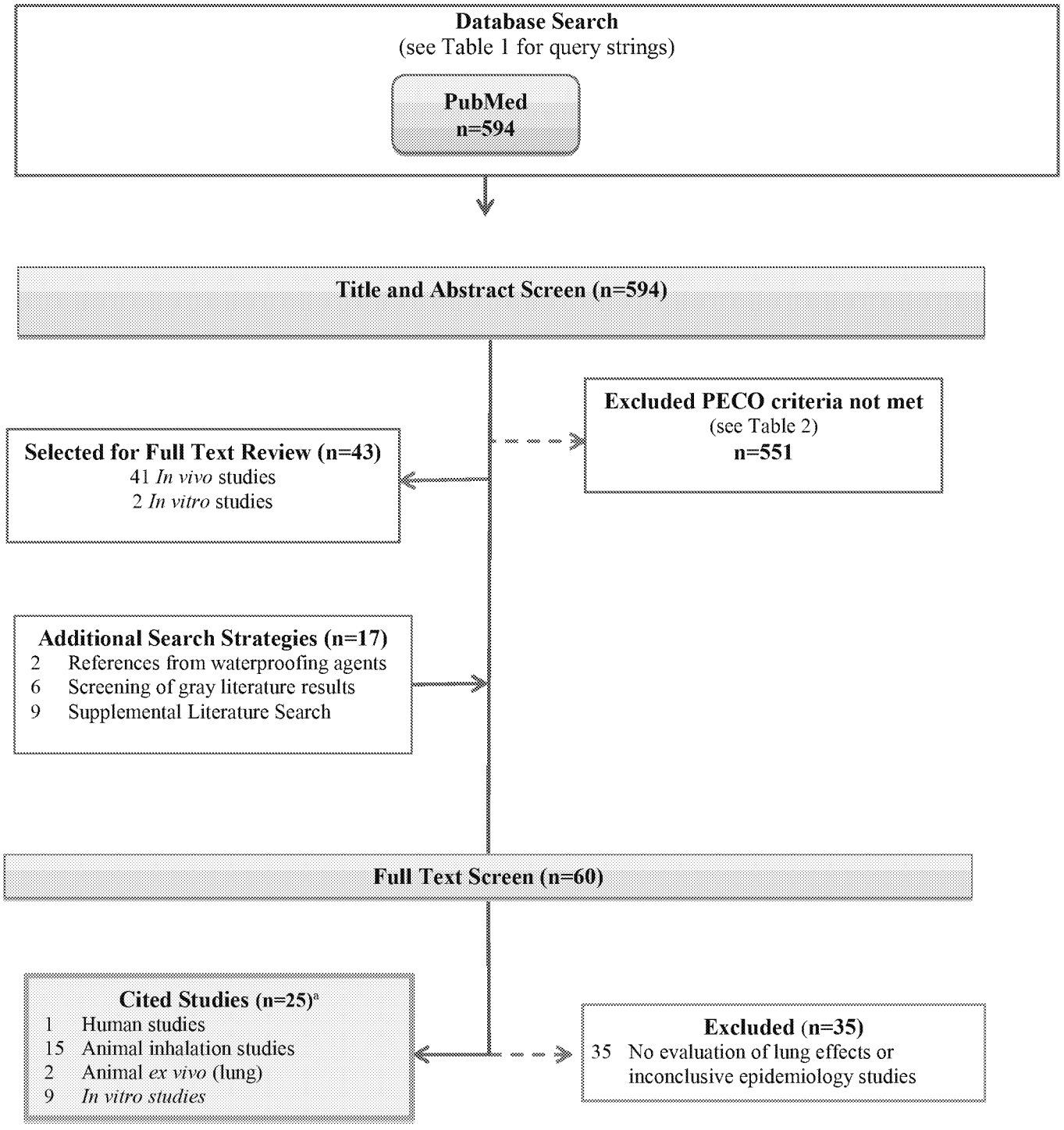

Figure [SEQ Figure * ARABIC]. Literature search and screening flow diagram for surfactants. [a] Two studies had both animal and *in vitro*/*ex vivo* data.

B. Supplemental Literature Search

i. Search Strategy

To identify hazard concerns associated with inhalation exposure to general surfactants, the search strings presented in [REF _Ref46547800 \h * MERGEFORMAT] and [REF _Ref46547863 \h * MERGEFORMAT] were used for PubMed and Embase, respectively, to be more comprehensive. The results for this review are presented in [REF _Ref46548065 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. PubMed Search strategy for general surfactants.

("surface-active agents"[mh] OR ((cationic OR anionic OR nonionic OR aerosols[mh]) AND surfactant*) OR detergents[mh] OR "pulmonary surfactants"[mh]) AND (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]) AND ("in vitro" OR "inhalation exposure"[mh] OR inhalation[mh] OR ((exposure OR administration) AND (intratracheal OR intranasal OR inhalation*))) AND English[lang]

Table [SEQ Table * ARABIC]. Embase Search strategy for general surfactants.

('surfactant'/exp OR ((cationic OR anionic OR nonionic OR 'aerosol'/de) AND surfactant*) OR 'detergent'/de OR 'lung surfactant'/exp) AND ('lung disease'/exp OR 'cell respiration'/exp OR 'surface tension'/exp) AND ('in vitro' OR 'inhalation'/exp OR ((exposure OR administration) AND (intratracheal OR intranasal OR inhalation*))) AND [embase]/lim NOT ([embase]/lim AND [medline]/lim) AND [article]/lim

ii. Study question and PECO criteria

The study objective was to identify physical/chemical properties and toxicity characteristics of substances that fall into the general surfactants chemical category and result in acute pulmonary toxicity following inhalation exposure. The study question was:

> What are the physical/chemical properties and toxicity characteristics of substances that fall within the general surfactants category and result in acute pulmonary toxicity following exposure *via* inhalation?

A study reported in the peer-reviewed literature was determined to be relevant and selected for full-text review, or excluded, based on the PECO criteria outlined in **[REF _Ref46548160 \h * MERGEFORMAT]**, in which study populations, study design, comparison groups, and measured outcomes are identified. The studies identified for full-text review were not scored for quality, but were reviewed with quality in mind to provide critical information that supports a mode of action for effects of surfactants in the lung. The exposure levels at which toxicity occurs, along with responses that may be influenced by factors such aerosol droplet size, were indicated as relevant information to capture for addressing the study question.

Table [SEQ Table * ARABIC]. PECO criteria for general surfactants.

Population	Humans and animal in vivo models or in vitro models using lung tissue slices or cells. Exclude: unhealthy human populations; disease-induced experimental animals.
Exposure	Inhalation exposure (including intratracheal and intranasal administrations) to general surfactants.
Comparator	No exposure, room air exposure (animal studies), or vehicle control (including intratracheal and intranasal administration and in vitro studies).
Outcome	Properties of general surfactants associated with acute pulmonary toxicity resulting from surfactant effects on cell membranes that could alter pulmonary function, with specific attention to exposure concentration and duration to identify effect levels.

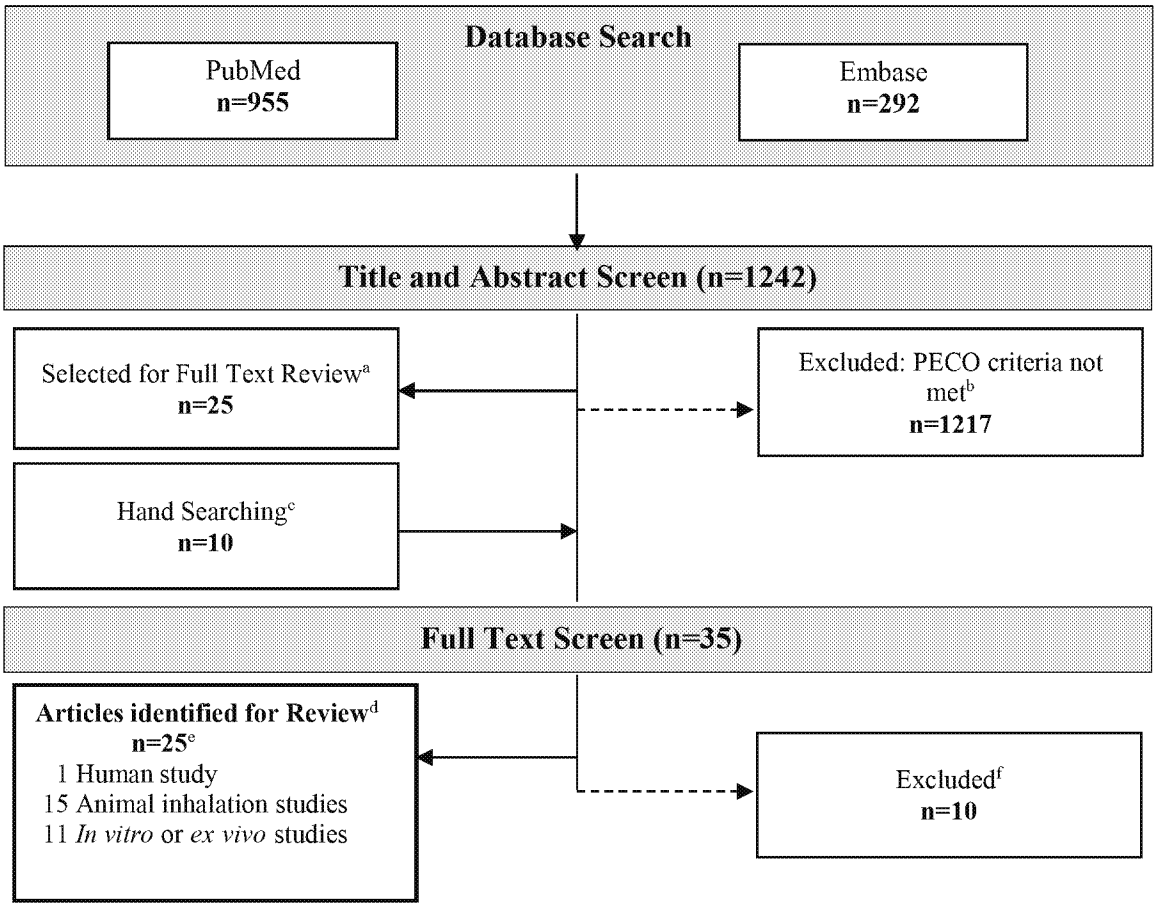

Figure [SEQ Figure * ARABIC]. General surfactants: search strategy and results. [a] Selected based on title and abstract screen; [b] Excluded based on title and abstract screen; [c] Identified by hand-searching, either found in articles reviewed, or identified in the Initial Literature Search; [d] Studies identified as relevant for integrating into hazard summary; [e] two studies had both animal and *in vitro*/*ex vivo* data; and [f] Key studies and review articles saved and used for contextual information are listed separately in the reference list.

C. Hazard concerns

Dysfunction of the pulmonary surfactant is a concern, considering that exogenous surfactants can damage the pulmonary surfactant resulting in impaired pulmonary function. This effect has been observed in human volunteer studies and in animal models. The older studies in the literature that focused on damage to the pulmonary surfactant were driven, in part, by a condition referred to as adult respiratory distress syndrome (ARDS) (reference to this cited by Nieman et al., 1990). When the clinical symptoms associated with ARDS are severe, there is alveolar flooding with protein-rich fluid. As described by Nieman et al. (1990), alveolar epithelial permeability is unchanged in early ARDS but changes occur in later stages, as a result of proteinaceous fluid entering air spaces through the bronchiolar epithelium. Because plasma proteins can inhibit surfactant function and increase surface tension and epithelial permeability, studies were initially carried out with inhaled aerosol detergents to study the mode of action of ARDS in animal models.

The hazard concern associated with inhaled general surfactants is that damage to the pulmonary surfactant results in an increase in surface tension within the lung, thereby affecting oxygen transfer. These concerns stem from:

- Dysfunction of natural surfactant in the lung from inhalation of substances with surfactant properties.

- The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function (demonstrated in human volunteers and in laboratory animals).
- The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which, are likely to vary based on the chemistry of the surfactant and the exposure method (*e.g.*, aerosol droplet size).

A total of 25 references were identified for full-text review from both literature searches and screening efforts. One human study was removed from the Supplemental Literature Search as it was only available in the Japanese language. A tabular summary of the 24 peer-reviewed publications that provide critical information for this evaluation is provided in [REF _Ref46836960 \h]. The study summaries with respect to the PECO criteria identified in this review are provided below by study category (*i.e.*, human and animal *in vivo*, and *in vitro* or *ex vivo*) and are summarized with general comparisons to the findings from the Initial Literature Search. A number of articles identified by hand-searching were captured and reviewed, but they did not meet the PECO criteria (*e.g.*, reviews or studies), so were held and reviewed for contextual information.

Table [SEQ Table * ARABIC]. Summary of peer-reviewed articles identified for full text review.

Author/Title	Defined test substance	Study type / Model	Exposure route / concentrations	Study description	Aerosol / particle size	Outcomes / Toxicity	Authors' conclusions
Bachofen et al., 1979. Alterations of mechanical properties and morphology in excised rabbit lungs rinsed with a detergent.	Triton X-100	*Ex vivo*, isolated perfused lungs, rabbit	Alveolar lavage, 0.01% solution in Triton X-100 in saline - Comparator was baseline, N	Alveolar lavage in isolated perfused lungs. Degassed lungs inflated 0.01% detergent solution to peak pressures of 10-15cmH_2O and deflated to 0 (3x). About 5 ml of solution remained in lungs following procedure. Measured total lung capacity, PV curves, fixed lung tissue and performed morphological evaluation.	N/A	Total lung capacity: progressive collapse of alveoli, with most alveoli collapsed at 40% TLC; Pressure-volume curves: Triton-rinsed lungs had a shift of the deflation limb to the right; Morphological evaluations: no gross effects on alveolar septa, some localized damage of squamous alveolar epithelium, focalized collapsed areas, with macrophages.	Hence, the results indicate that in detergent-rinsed lungs volume changes are brought about predominantly by recruitment and derecruitment of alveoli. It appears that both a normal surfactant and the mechanical interdependence within the fibrous continuum are required to maintain a normal respiratory surface area within the lung volume range of normal breathing.
Damon et al., 1982. Acute toxicity of polyethylene glycol p-isooctylphenol ether in syrian hamsters exposed by inhalation on bronchopulmonary lavage. **Identified in Initial Literature Search**	Polyethylene glycol p-isooctylphenyl ether (Triton X-100)/3H-Triton X- 100	*In vivo*, male and female Syrian hamster	1) nose-only inhalation (nebulizer) ethanol only 2) aerosol 10% Triton X-100 in ethanol , 0, 800, 1400, 1900, 2500 in 800-3100 ug estimated lung burden 3) lungs lavaged (instillation) with 0.01, 0.05, 0.06, 0.075, 0.10% Triton X-100 solution in saline (lung burden = 300-3200µg)	Hamsters exposed *via* nose-only inhalation and removed in groups of 10 at different time intervals to provide intial respiratory tract burdens (RTB) ranging from 800- 3100µg. A second group was exposed in a similar fashion to an aerosol to provide similar RTB. For bronchial lavage, hamsters were injected 2x with 0.01-0.10% in isotonic saline. Animals were placed on 100% oxygen until normal breathing was restored. Mortality of the hamsters was analyzed through day 7.	Nose-only inhalation: MMAD = 1.47±0.06 µM, GSD = 1.84 ± 0.07, mass concentration of 3.0 mg/liter; or an aerosol with MMAD = 1.51 ± 0.07µM, GSD = 1.91±0.08, mass concentration of 3.0 mg/liter	General observations: lavaged hamsters displayed wheezing and dyspnea, the nose- only inhalation groups displayed inspiratory and expiratory dyspnea and matting of hair from nasal and oral fluids. Mortality: in the lavage study, a progressive increase in mortality was observed with increasing dose of detergent. In the inhalation study, the mortality increased with increasing lung burden. LD_{50}/7s did not significantly differ between the inhalation and lavage studies. Pathology: lung congestion, focal areas of peripheral atelectasis and blood-tinged fluid were noted in the lavage groups. Hamsters that died early (<1hr) showed sever intraseptal and peribronchial congestion. At	Histopathological examinations revealed differences in the nature and distribution of pathologic changes observed in animals exposed by the two routes of administration. Animals exposed by inhalation died as a result of ulcerative laryngitis and laryngeal edemal compared to those exposed by lavage, which died from pulmonary edema and acute exudative pneumonia. One might speculate that the respiratory tract damage observed in these studies is due to initial disruption of epithelial cell membranes followed by an inflammatory reaction to the necrotic cells. Certainly, the histological sequence described above is consistent with such a mechanism of injury.

						1-5 hrs alveoli and terminal bronchioles contained large # of neutrophils. By 24hs, changes were more diffuse and exudate contained neutrophiles and macrophages as well as cellular debris. In the inhalation studies, all spontaneous deaths occured by day 6. Hamsters displayed laryngeal and epiglottic edema, and edema resulted in reduced diameter of laryngeal lumen. Death was attributed to obstructive asphyxia. Mucosal ulcerations of the laryngeal sections had neutrophils and macrophages.	
Ehrhart et al., 1981. Oleic acid dose-related edema in isolated canine lung perfused at constant pressure.	Oleic Acid	*Ex vivo*: isolated dog lungs	Oleic acid dosage µl/kg 0, 1, 45	Isolated perfused at constant pressure with heparinized blood with exposure to various concentrations of oleic acid in the perfusate. Weights changes and electrically averaged vascular pressures were continuously measured. Blood flow was measured by timed collections.	N/A	Weight gain increased linearly over 1-3 h following oleic acid with regression slopes indicating a more rapid rate of weight gain at the higher oleic acid dosage. Total lobe weight gain was greater in the 45 versus 1 µl/kg group. Pulmonary vascular resistance increased at 45 µl/kg oleic acid but was unchanged at 1 ul/kg oleic acid or saline. The decrease in arterial O2 partial pressure was greater in the 45 µl/kg group than in the controls, 47 versus 22 Torr.	"Weight gain related to oleic acid dosage suggests that oleic acid increases permeability by affecting the vascular endothelium either directly or through biochemical intermediates endogenous to the lung or blood."
Ekelung et al., 2004. Correlation between epithelial toxicity and surfactant structure as derived from the effects of polyethyleneoxide surfactants on Caco-2 cell monolayers	Surfactants are abbreviated CnEm: n is the number of carbons in the saturated hydrocarbon chain, m is the number of repeating ethylene oxide units in the head group. Parentheses-	*In vitro*: Tensiometer to measure surface tension, and effects on Caco-2 cells in pig nasal mucosa in Using chamber experiments.	Incubation with concentrations ranging from 10e-5 to 10 mM	Caco-2 cells- to measure TEER Surfactant effects on transport of radiolabeled mannitol, testosterone, or propranolol. Using chamber experiments; isolated nasal respiratory mucosa from domestic pigs	N/A	Surface tension of PEO alkyl ethers decrease with increasing alkyl chain length in the homologous surfactant series. After 1-hr incubation in Caco-2 cells all surfactants showed concentration-dependent effects on TEER. TEER decreased over a narrow concentration interval with marked increased in mannitol permeability and trypan blue accumulation.	"The concentration-dependent effects of two series of homologous nonionic surfactants on Caco-2 cell monolayers and pig nasal mucosa have been studied. A correlation between surfactant molecular structure and adverse epithelial effects showed the size of the hydrophilic head group to be more critical than the hydrocarbon chain length. All surfactants tested, except C12E8 and C12Eh23i, could be used at concentrations above cmc without having any adverse effects on the TEER of the

and pig nasal mucosa.	average numbers used for surfactants that are polydispersed with response to the PEO chain, no parentheses, surfactant is regarded as being monodispersed. C12E8, C12E(23) (BRIG 35), C14E8, C16E8, C16E(20) (BRIG58), C18E(20) (BRIG 78), M-C18E(20) (MYRJ 49), M-C18E(40) (MYRJ 52), M-C18E(100) (MYRJ 59)						Caco-2 cell monolayers. The trends found in the Caco-2 study were confirmed by in vitro experiments on pig nasal mucosa mounted in a horizontal Using chamber. However, the nasal mucosa could be exposed to somewhat higher surfactant concentrations without being affected, suggesting mucus to act as a protective barrier. Altogether, the results are highly relevant for rational selection of PEO surfactants that combine a high solubilizing capacity with a low local toxicity. Combining the data from the study of budesonide solubilization with those from the cell studies showed M-C18Eh40i to be an efficient solubilizer, at concentrations where we observe no detrimental effects on cells. In more general terms, the data in this work strongly suggest that surfactants with long PEO head groups are less toxic than analogs with short PEO groups. This, in turn, suggest that micellar surface absorption, together with bulk micellar solubilization, are two critical steps in the process of solubilization of membrane constituents."
Evander et al., 1988. Pulmonary clearance of inhaled ^{99}mTc-DTPA: effect of the detergent dioctyl sodium sulfo-succinate in aerosol.	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbit	Aerosol inhalation - 5% solution in saline for 5 minutes	5 min inhalation of saline or DOSS followed by ^{99}mTc-DPTA via aerosol. $_{P}O_2$, $_{P}CO_2$ and clearance of ^{99}mTc-DPTA measured.	Not given but likely 1.7 µM by air jet nebulizer.	Increased clearance of Tc-DPTA; no effect on pressure or compliance.	Clearance of Tc-DTPA is increased with DOSS through interference with surfactant, not through alveolar capillary disruption.

Evander et al., 1994. Biexponential pulmonary clearance of 99mTc-DTPA induced by detergent aerosol. **Identified in Initial Literature Search**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbit	Aerosol inhalation - 0, 0.125, 0.25, 0.5, 2%	Protocol A: ultrasonic nebulizer used to suspend 2, 0.5, 0.25 or 0.125% DOSS solution, exposure for 5 min through ventilator. Immediate aerosol treatment with ^{99}mTc-DTPA (3.3 µm particle size). Protocol B: 2 or 0.5% DOSS solution, exposure for 5 min through ventilator. After 60 min, aerosol treatment with ^{99}mTc-DTPA (3.3 µm particle size). Protocol C, ^{99}mTc-DTPA started before DOSS. Arterial blood gases, tidal volumes, airway pressure recorded at 90 and 180 minutes. Evaluation of ^{99}mTc-DTPA clearance also evaluated.	DOSS MMAD = 1.7µM; ^{99}mTc-DTPA MMAD = 3.3 µM	No effect on blood pressure, PaO_2, $PaCO_2$, or compliance (Crs). Biphasic clearance of Tc-DTPA observed after DOSS exposure, but not with vehicle control. Fast clearance followed by slow clearance. % eliminated in fast phase was dose-dependent. $T_{1/2}$ slow may show saturation, but $t_{1/2}$ fast was constant at high doses.	In summary, we have demonstrated that dioctyl sodium sulfosuccinate induces a biexponential clearance course of ^{99}mTc-DTPA by accelerating the transfer of the tracer across the alveolocapillary barrier in a separate pool of lung units, the size of which is dependent on the dose of detergent. The effect of detergent is partly reversible and may be caused by surfactant dysfunction.
Fischer et al., 2012; A pilot study on the refinement of acute inhalation toxicity studies: the isolated perfused rat lung as a screening tool for surface-active substances. **Identified in Initial Literature Search**	Agents tested but not identified; 12 surfactant active substances - 12 different waterproofing agents - 12 fluorocarbon molecules with side chains with 4-carbons (#1, 8), 6-carbons (#7, 9, 11), 8-carbons (#2-6, 10, 12) and solvent control	*In vitro*, isolated, perfused lung (IPRL) removal of the heart-lung block (male and female Wistar rats) to thoracic chamber and ventilated artificially at 80 breaths/min under negative pressure. Compared to *in vivo* results	Exposure using a compressed air-jet nebulizer - resulted in a reduction in particle size. Aerosolized pure solvent (n-hexane) as control, then 500 µl spray formulation over 10 sec was delivered to lung. After an hour, up to 10 bolus applications were performed, separated by a min. Then a third sequence of 20 boluses	Complex. Multiple short exposures with multiple recovery periods. Doses calculated from concentration, ventilation rate, time, *etc*. Measure tidal volume, resistance, compliance edema, mortality.	Not given	IPRL parameters included respiratory, atelectasis and reversibility. The acute inhalation toxicity test induced breathing pattern and pathology. Toxicity included lethality, pulmonary hemorrhage and edema, inflammation (increased erythrocytes/leukocytes in BALF), tachypnea, Alveolar type 1 cell cytotoxicity. The more toxic formula had increased fluoroalkenes, fluorophenyl and fluoroalcohol WPAs, but also had addition of 2-butoxyethanol (which is toxic) and dipropylene glycol methyl ether and C10-12 alkanes.	IPRL model correlates well with *in vivo* acute inhalation toxicity (OECD TG 403 at 20 mg/L limit concentration)

		(OECD 403)	after one hour. Exposure dose - 45-3125 µg/lung				
Hall et al., 1992. Inhibition of pulmonary surfactant by oleic acid: mechanism and characteristics	Oleic acid	*In vitro*, surfacto-metry; *ex vivo*, perfused rat lungs	Instillation with 4, 10, or 20 mg OA dispersed by sonication in 2 ml saline.	In excised lung experiments, after excision and degassing, lungs were inflated to 30 cm H_2O and underwent stress relaxation for 10 mins (2x). Baseline PV characteristics and TLC were determined. Surface tension was measured with an oscillating bubble surfactometer, adsorption measurements and surface-tension- lowering characteristics were investigated with a Wilhelmy balance	N/A	Disruption of surfactant activities and lowering of surface tension. OA did not inhibit the adsorption of NLS but did form miscible interacting films with DPPC. In excised rat lungs, OA progressively shifted curves, producing significant further volume loss at lower pressures, at increasing doses. Oleic acid inhibited pulmonary surfactant activity by disrupting the rigid interfacial film that maintains low surfactant tension. Instillation of oleic acid resulted in altered deflation pressure-volume characteristics suggestive of an effect on pulmonary surfactant.	The detrimental mechanical alterations induced by treatment of excised lungs with OA must reflect changes in the interfacial function of pulmonary surfactant induced by the fatty acid. Oleic acid mixes with surfactant and impedes function of surfactant - destabilizes surface film during dynamic compression.
Jeffries et al., 1988. Effect of increased surface tension and assisted ventilation on ^{99}mTc-DTPA clearance.	Dioctyl sodium sulfosuccinate (OT)	*In vivo*, New Zealand White rabbit	Inhalation, aerosol of 20 mL 1.5% solution	Rabbits, inhalation of aerosol - 20 mL 1.5% solution for 20 minutes followed by ^{99}mTc-DTPA aerosol for 1-2 mins with free breathing, conventional ventilation, or high frequency oscillation ventilation.	Aerosol contained particles with aerodynamic mass median diameter = 0.6 µM and GSD = 1.97 µM	Clinical signs of respiratory distress were noted in all rabbits administered OT. Acidosis and declining oxygenation increased with time following administration. Spontaneously breathing and CMV groups had increasing $PaCO_2$, with statistical significance in CMV animals. Foam was present in the trachea, small airways and on the cut lung of animals at the end of the experiment. Lung volume (pressure- volume curve) was decreased in rabbits treated with DOSS compared to vehicle treated. ^{99}mTc-DTPA clearance significantly increased in animals treated with DOSS, regardless of modes of	A change in the surface tension properties of the lung as a result of detergent administration results in an accelerated clearance of the small solute ^{99}mTc-DTPA, suggesting an increase in the permeability of the pulmonary epithelium.

						ventilation.	
John et al., 1997. Additive nature of distension and surfactant perturbation on alveolocapillary permeability. **Identified in Initial Literature Search**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbits	Inhalation, aerosol of 2% detergent	Rabbits were exposed to vehicle or DOSS *via* conventional or large tidal volume ventilation followed by a recovery period. ^{99}mTc-HSA was administered following exposure and clearance was measured during 3 hours of conventional or LTVV. Vehicle or DOSS administration was repeated 90 minutes after ^{99}mTc- HSA administration. Lung mechanics and arterial blood gas determination were evaluated.	N/A	DOSS decreased the half-life of clearance ($t_{1/2}$). At necropsy, only animals in the detergent + LTVV group had foam in the trachea and on cut lung surface.	In conclusion, the mechanisms of an increase in clearance during lung distension related to large tidal volume ventilation and perturbation of the surfactant system with detergent are different, as seen from the distinct nature of their clearance kinetics. When these mechanisms are combined, they display additive features. Either of the individual mechanisms related to detergent or large tidal volume ventilation is reversible. However, a combination of detergent and large tidal volume ventilation leads to nonreversible changes in lung function and lung injury.
Martinez & Brown, 1991. Oral and pulmonary toxicology of the surfactant used in roundup herbicide.	Polyoxyethylene amine (POEA) or Polysorbate-80, non-ionic surfactants	*In vivo*, rats	Test agent administration directly into trachea; POAE (7%) at 0.1, 0.2, and 0.4 mL, and polysorbate-80 (7%) 0 at 0.1 and 0.2 ml.	Post administration (24 hr) lungs were dissected and lung weight and subjective scaling of lung damage was scored.	N/A	Following tracheal administration of POEA, (7%) produced 20, 70, 100% death at 0.1, 0.2, and 0.4 mL, respectively, increased lung weight and lung damage (subjective scoring) whilepolysorbate-80 did not produce any deaths, had no effect on lung weight or visible lung damage.	"The present experiment shows that the non-ionic surfactant, POEA, has serious pulmonary toxicity although not as much as the Roundup combination. In comparison, polysorbate-80, a non-ionic pharmaceutical surfactant, had little significant pulmonary effects except at the highest dose. Neither POEA or PS-80 produced any significant pulmonary injury or death when given orally at doses of up to 1.03 g/kg (5 mlx0.07/0.340 kg rat)."

Meinert et al., 1992. Syntheses, interfacial active properties and toxicity of new perfluoroalkylated surfactants.	9 Different perfluoralkylated surfactants- with same fluorophilic tail and hydrophilic heads but different prolongators.	*In vitro*, interfacial-tensiometer Lecomte du Nouy method using a rigid platinum ring. Toxicity evaluated in HeLa cells (epithelic cells from cervix) and Molt 4 cells (T-cell leukemia cell line)	Surfactants dissolved in isotonic buffer (10% w/v) were identified as % (w/v) in culture (0.04 to 2.5).	Measured surface tension and interfacial tension water/perfluorodecalin were measured, CMC (critical micelle concentrations) was calculated. Biocompatibility test was used using cell proliferation (^{3}H-thymidine incorporation) as the measure.	N/A	In the cell cultures, surfactants caused a significant reduction in proliferation depending on the concentration and chemical nature of the agent. One surfactant, caused > 50% inhibition produced by concentrations greater than 0.16% in both cell lines. Note; no direct correlation of biocompatibility with surface tension or interfacial tension. was observed	"Interestingly, the b-series of surfactants (containing a $(C_2H_{40})_1$ $2CH_3$- group) were in general less biocompatible than surfactants of the a- series. For the surfactants under test, number IVa, containing a $(CH_2H_{40})_7$ CHj-group, seems to be the one with the best biocompatibilty. According to our experiments this component is at least equal to or better than Pluronic F68. Obviously, there is no direct correlation of biocompatibility never with surface tension nor with interfacial tension H20/PFC. It seems, that a branched prolongator promotes biocompatibility of a surfactant more than an unbranched one."
Modell et al., 1969. The effects of wetting and antifoaming agents on pulmonary surfactant. **Identified in Initial Literature Search**	Alevaire	*In vitro*, Wilhelmy balance to measure surface tension using pulmonary surfactant extracted dogs and *In vivo* studies in adult dogs	*In vitro*: Normal saline versus Alevaire (1 to 30 mL added to 150 mL of saline) / Ethyl alcohol (1 to 100 ml in 150 mL of saline) *In vivo*: endotracheal catheter pass *via* a tracheostomy for measurements from one lung, and ultrasonic nebulizer with Alevaire or ethyl alcohol continuously for 8 hours	*In vitro*: measures of surface tension with exposure *In vivo*: measures of blood gases, alcohol concentrations in blood and breathed aerosol prior to and post 2, 4, 5, and 8 hours of exposure. Surface tension of lung measured.	N/A	*In vitro*: No concentrated-related differences in surface tension - surface area loop., but there was a progressive decrease in the surface compressibility of the film (*i.e.* narrowing hysteresis loop) that were then reversed. *In vivo*: There was a significant decrease in arterial oxygen tension with aerosol and a slight decrease in $PaCO_2$ with corresponding increase in pH. Surface tension- surface area loop showed normal hysteresis in all cases, unlike observed *in vitro*.	"Our results prove that both wetting agents and antifoaming agents can change the surface tension-surface area loops recorded on compression and expansion of normal pulmonary surfactant. This phenomenon is concentration-dependent, however, and small quantities of either of the two substances can be present without altering surface tension." "The concern that these agents will alter surface tension at the air-liquid interface and result in unstable alveoli and atelectasis when used for a reasonable period of time does not appear justified. A more likely hazard with continued use is the accumulation of fluid in dependent areas of the lung, resulting in intrapulmonary shunting and hypoxia."

Nieman et al., 1985. High surface tension pulmonary edema induced by detergent aerosol. **Identified in Initial Literature Search**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, mongrel dog; *in vitro*, minced lung extracts	Inhalation, aerosol *via* ventilator, 15 mg/kg in 1% solution	An ultrasonic nebulizer was used to suspend 1% solution, a total volume of 1.5 ml/kg was administered over 30-45 min through ventilator. Surface tension measured with Wilhelmy balance using lung extract and tissue from lung at 4h post-exposure. Airway foam from distal trachea or large bronchii was similarly tested. The study measured arterial pressure (femoral, pulmonary), blood gasses, hemoglobin and blood pH. pulmonary extravascular water volume was studied. Microscopic examinations were performed and edema assessment (pulmonary extravascular water volume) measured by gravimetric technique. For the *in vitro* study, lung samples were taken 30 and 120 minutes after aerosol inhalation.	Mean = 3 µM (range, 0.5- 15 µM)	Partial diffuse lung collapse worsened over time accompanied by a progressive decrease in lung volume at end of expiration. Edema fluid appeared as foam in small airways (following lung collapse), by 2 hr extensive foam in the major bronchii and distal trachea were noted. Destabilization and large changes in size of subpleural alveoli were observed. Decreased surface tension. Diminished surfactant activity measured by Wilhelmy balance. Compared to controls, PEWV increased (extravascular water volume) in animals killed 2 hours following aerosol administration.	The sequence of events, with the evidence of alveolar instability appearing prior to edema, implies that the loss of alveolar surfactant is initiating subsequent events rather than occurring later as a nonspecific consequence of edema formation. We thus conclude that the increase in PEWV is the result of the displacement of surfactant by detergent and the consequent increase in alveolar surface tension as originally predicted by Pattle (21) and Clements (7).
Nieman et al., 1990. High alveolar surface tension increases clearance of technetium ^{99}m diethylenetriamine-pentaacetic acid. **Identified in Initial Literature**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, mongrel dog	Inhalation, aerosol *via* ventilator, 15 mg/kg in 1% solution	An ultrasonic nebulizer was used to suspend 1% solution, a total volume of 1.5 ml/kg was administered over 30-45 min through ventilator. After delivery of DOSS, an aerosol of 99mTc-DTPA (particle size <1µM, diethylenetriaminepentaacetic acid) was administered via inhalation over 5 min. Effects studied continuously over 4 h. Measured arterial pressure, blood gasses, and	Mean = 3 µM (range, 0.5 - 15 µM)	Arterial O_2 tension decreased and peak airway pressure increased following treatment. ^{99}mTc-DPTA clearance (decreased $t_{1/2}$) was significantly faster in treated animals compared to controls.	In summary, we have shown that elevating alveolar surface tension accelerates the clearance rate of aerosolized ^{99}mTc-DTPA. It is remotely possible that the surfactant layer is a barrier to ^{99}mTc"DTPA diffusion and that removal of this layer accelerates solute flux. More likely, high alveolar surface tension increases epithelial permeability as a result of regional hyperexpansion. The resultant increase in solute flux suggests that surfactant deactivation by plasma proteins originating from the bronchiolar epithelium, in the early stage of

Search				clearance of TC- DTPA to evaluate permeability of lung epithelium.			ARDS, represents a plausible mechanism for the later alveolar flooding commonly seen clinically and radiographically
Nilsson et al., 1992. Pulmonary clearance of ^{99}mTc-DTPAand ^{99}mTc-albumin in rabbits with surfactant dysfunction and lung injury. **Identified in Initial Literature Search**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbit	Inhalation, aerosol via ventilator, 1% solution, dose not noted	Rabbits were treated with aerosolized ^{99}mTc-DTPA or ^{99}mTc- albumin and monitored for clearance for 30 min. A subsequent treatment with aerosolized DOSS for 5 minutes was monitored for another 30 minutes followed by an i.v. injection of oleic acid (0.17 ml/kg). Clearance was measured again 30 minutes later. Second set of rabbits treated with ^{99}mTc-DTPA and administered DOSS aerosol or oleic acid injection 30 minutes later. Clearance was measure for another 30 minutes. Clearance of ^{99}mTc-DPTA, arterial pressure, PaO_2, and $PaCO_2$, were evaluated.	N/A	TC-albumin clearance slightly lower (not significant) with DOSS, and much lower with DOSS + oleic acid. ^{99}mTC-DTPA clearance was significantly lower than control with either DOSS or oleic acid. DOSS alone did not affect PaO_2, $PaCO_2$ or compliance, however administration of oleic acid resulted in a reduction in PaO_2 and an increase in $PaCO_2$	The findings in this study indicate that surfactant dysfunction induced by detergent does not appreciably affect the alveolocapillary transfer of proteins, while the more extensive injury caused by oleic acid increases the clearance of proteins. The findings may be explained if different components of the alveolo-capillary membrane are regarded as serial barriers. Thus, damage to the surfactant barrier may not lead to increased alveolo-capillary transfer of Tc - albumin if the epithelial barrier is left intact. The epithelial barrier may be considerably more permeable to Tc- DTPA than to Tc-albumin.
Nilsson et al., 1993. Pulmonary clearance of tracers with different lipid and water solubility in experimental surfactant dysfunction. **Identified in**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbit	Inhalation, aerosol *via* ventilator, 1% solution, dose not noted	Surfactant dysfunction was induced by administration of DOSS aerosol for approximately 5 minutes *via* ventilation. The DOSS aerosol was followed by an immediate intratracheal instillation of ^{99}mTc-DTPA, ^{99}mTc-sestamibi, or ^{99}mTc- HIDA. Clearance of radioactives, airway pressure, dynamic	N/A	Clearance of ^{99}mTc-DPTA was substantially increased following DOSS administration, but only slightly for ^{99}mTc-sestamibi. No difference was seen in clearance of ^{99}mTc-HIDA. DOSS had no significant effect on PaO_2, $PaCO_2$, and Crs in any group.	The rank order of the detergent effect was inversely related to the rank order of the lipid/water partition coefficient, (so detergent affects transfer of hydrophilic compounds more). This study has shown that the rate of pulmonary clearance is faster for very lipid soluble substances than for water soluble substances with similar molecular radius and weight. The clearance rate of very lipid soluble tracers is not, or is only slightly,

Initial Literature Search				compliance, and blood gasses were evaluated.			affected by the surfactant dysfunction. Thus, the surfactant system seems to affect the transfer of small water-soluble molecules but not the transfer of substances with high lipid solubility.
Nilsson et al., 1997. Pulmonary clearance of ^{99}mTc-DTPA in experimental surfactant dysfunction treated with surfactant instillation. **Identified in Initial Literature Search**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbit	Inhalation, aerosol via ventilator, 2% solution for 5 minutes	Induced surfactant dysfunction with DOSS aerosol (approx. 5 min ventilation), resulting in approximately 10 µl of fluid in the lungs, followed by immediate intratracheal instillation of saline or natural (bovine) surfactant. ^{99}mTC- DTPA was administered as an aerosol via ventilation circuit. ^{99}mTc-DPTA clearance was measured 30 min after treatment. Airway pressure, blood gasses, and lung morphology were evaluated.	MMAD = 1.7 µm	Animals treated with DOSS, with and without surfactant treatment, displayed decreased oxygen tension, decreased compliance, decreased $T_{1/2}$ (increased permeability) of ^{99}mTc-DTPA. Surfactant treatment significantly attenuated the effect but did not restore normal functions. Morphology of control experiments with DOSS alone showed minor injury with alveolar expansion, pulmonary edema, injury to airway epithelium and inflammation.	In summary, in agreement with the hypothesis, tracheal instillation of natural surfactant markedly attenuated the effect of detergent on the pulmonary clearance of ^{99}mTc-DTPAT. This clearance model may be used to optimize the technique of surfactant administration and also to evaluate the clinical effect of the treatment.
Obenour et al., 1963. Effects of surface- active aerosols and pulmonary congestion on lung compliance and resistance. **Identified in Initial Literature Search**	Defomaire	*In vivo*, human	Inhalation, *via* nebulizer, 3 mL	Normal healthy volunteers were administered 3 mL siliconized respiratory detergent via nebulizer during a 6-minute period. Lung compliance was determined by measuring the volume and intrathoracic pressure changes for each respiration at a time of zero airflow velocity. Pulmonary resistance, was calculated using a value representing the sum of airway and lung tissue resistance.	N/A	Pulmonary compliance significantly decreased, and tissue resistance significantly increased following nebulized Defomaire.	In the present studies, we have attempted to demonstrate surface tension phenomena by observing the effect of surface-active aerosols upon pulmonary compliance and resistance. In order to relate surface tension to the mechanics of breathing, the Laplace equation has been used after making the assumption that the alveolus has the physical properties of a bubble.17-19 Simply stated, this relationship means that the internal pressure of a bubble is directly proportional to twice its surface tension divided by its radius. If this relationship is true for the lung, an agent that lowers surface tension in the alveoli should cause an increase in

							compliance, since less pressure would be required for maintenance of any given volume. The converse would also be true. Our compliance data for alcohol is consistent with such a theory. "Although nebulizations do not penetrate pulmonary tissues in a complete or uniform manner, comparable aerosols have been demonstrated to enter the alveolar air spaces and pulmonary circulation in significant quantities."
Rao and Das, 1994. Pulmonary oedema due to inhalation of detergent aerosol.	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, male Wistar rat	whole-body inhalation, aerosol, 100 (2 ml), 200 (4 ml), 300 (6 ml), 400 (8 ml), or 500 mg (10 ml) of detergent	Whole-body inhalation occurred for 10 minutes per 2 ml administered; *i.e.*, animals administered 100 mg (2 ml) were exposed for 10 minutes, while animals treated with 500 mg (10 ml) were exposed for approximately 1 hour. Animals were sacrificed and examined 30-minutes post-inhalation.	Nebulizer locally made and particle size could not be measured.	Pulmonary edema (bronchiolar and focal alveolar) was observed in 3/5 high-dose animals. Lungs were normal in all other animals.	It is possible that 500 mg of detergent aerosol is the minimum dose needed in these animals to interfere with surfactant activity leading to pulmonary oedema. The oedema could not be due to anaphylaxis to detergent or vehicle since none of the animals showed signs of any distress, and control animals did not have any pulmonary oedema. Hypoxia could not be a factor since the animals were breathing normally and a vent in the perspex chamber was opened now and then for circulation of air.
Sorli et al., 2015. An *In vitro* method for predicting inhalation toxicity of impregnation spray products. **Identified in Initial Literature Search**	1% POTS (hydrolysates and condensates of 1H,1H, 2H, 2H-perfluorooctyl-trialkoxysilan e in 2-propanol, product equivalent to non- absorbing floor materials - nine spray productes containing perfluoracrylate,	*In vitro*, capillary surfacto-meter; using bovine derived pulmonar y surfactant formulatio n Alveofact (contains phospholip ids and the	Alveofact (4 mg/mL) was incubated with the products diluted in original solvents or solvent alone. Dose of POTS (by volume) was added to mixtures, with solvents evaporated. The sample preparations	Inhibitory effects of these products on the pulmonary surfactant function was established for nine different products. The potency of the product for inhibition of surfactant function was evaluated based on highest concentration of POTS that did not have a significant inhibitory effect and then compared to previous published in vivo studies in mice that	N/A	All products that were toxic in mice exposed *via* inhalation (identified in Norgaard et al., 2010, 2014) inhibited the pulmonary surfactant function *in vitro*. Two products that were negative *in vivo* were negative *in vitro*. Two of three false positives were at the highest concentration. Negative predictive value was 100%; positive predictive value was 57%. 1) *in vivo*: "footwear protector" and "wood impregnation" caused an irreversible depression of tidal	"In conclusion, this study presents a proof-of-principle for using pulmonary surfactant inhibition as a predictor for toxicity of inhaled impregnation spray products in mice."

	alkylsilan / siloxan, perflurosilan / siloxan	hydrophobic pulmonary surfactant proteins SP-B and -C	were evaluated in a concentration dependent manner, however only by dilution, so actual concentrations are not provided.	evaluated acute pulmonary toxicity.		volume at 103 mg/m^3 and 114 mg/m^3, respectively, causing mortality in some but not all mice. "Rim sealer" caused irreversible depression of tidal volume in all mice at 1612 mg/m^3. None of the products caused upper or lower airway irritation. 2) in vitro: "Two products, “Textiles and leather” and “Special textile coating” had no inhibitory effect on pulmonary surfactant. The products “Car glass” and “Bath and tiles” had a high NOEL (>8% impregnation product), one product (“Rim sealer”) had a NOEL of 4%. Four impregnation products had low NOELs (<2%), two of these “Footwear protector” and “Wood impregnation” contained perfluoracrylate in a water and glycol solution. The remaining two products with a low NOEL contained perfluorsilan/siloxane in water (“Textiles and leather concentrate”) or 2-propanol (“Non-absorbing floor materials”). “Special textile coating” did not have an effect on the surfactant function, and “Rim sealer” had an inhibitory effect on the surfactant function.	
Taskar et al., 1996. Effect of detergent combined with large tidal volume ventilation on alveolocapillary permeability. **Identified in Initial Literature**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, rabbits	Inhalation, aerosol of 2% detergent	Rabbits were administered detergent or vehicle aerosol, followed by ^{99}mTc- DTPA, *via* a nebulizer, under conventional ventilation or LTVV. Clearance measurements were assessed for a 180-minute period. Lung mechanics (pressure and flow signals) and blood gases were	MMAD = 1.7 µM	There were no inter- or intra-group differences in arterial $_PO_2$ and $_PCO_2$ at baseline or final measurements. Final Crs was high and Pmean lower in the LTVV group versus the other three. Final Crs was lower and Pmean higher in the DOSS+LTVV groups, than those in the DOSS-only group. All animals in the DOSS+LTVV group had foam in the trachea and cut lung surface. ^{99}mTc-DPTA clearance was	In conclusion, we have demonstrated that the clearance kinetics of LTVV are qualitatively different from those of detergent. The effects of LTVV and detergent are additive. These mechanisms are probably additive because the kinetics of the combination of detergent+LTVV is characterized by a fast compartment similar in size to, but faster than, detergent, and a slow compartment similar

Search				measured following this 180-minute period.		bioexponential following DOSS administration with or without LTVV.	to LTVV.
Tsujino et al., 1999. Effect of Tween-80 on cell killing by etoposide in human lung adenocarcinoma cells.	Tween 80	*In vitro*, growth inhibition (A549, H69, PC14, PC14/CDDP, and KB cell lines)	Media, 100 and 250 µg/ml	A549 (human lung carcinoma), H69 (human small cell carcinoma), plus PC14, PC14/CDDP and KB cell lines. Cells were treated with etoposide, Tween 80, or etoposide + Tween 80. Survival was measured after 5 days.	N/A	Cell growth inhibition, increased uptake and accumulation of etoposide, but no change in uptake of hydrophilic compound daunorubicin was observed.	Owing to its lipotropic character, etoposide (VP16) might become more readily transported through the cell membrane by Tween-80, a surface- active agent. On the other hand, Tween-80 has been shown not to enhance VP16 accumulation in K562/S cells, in contrast to its effect in K562/ADM cells, because the effect of VP16 arises only at cell membranes already altered. On this basis, the membrane of lung adenocarcinoma cells is considered to have undergone modification beforehand (although the precise kind of change still remains unknown).
Wang et al., 1993. Influence of detergent aerosol on lung microvascular permeability. **Identified in Initial Literature Search**	Dioctyl Sodium Sulfosuccinate (DOSS)	*In vivo*, sheep	Inhalation, aerosol of 15 mg/kg DOSS in 30 mL of vehicle (saline + ethanol)	Sheep underwent 1 hr of vehicle or DOSS, followed by 12 hours of sampling and an additional 12 hours recovery. Sheep then received the other of the two treatments for 1 hour, followed by another 24 hours (sampling + recovery). The procedure was repeated with only a 2-hour recovery. Surface properties of bronchoalveolar lavage (Wilhelmy balance), PaO2, PaCO2 and pH were measured.	Mean = 3 µM (range, 0.5- 15 µM)	No change in PaO_2, $PaCO_2$, pH, with a small effect on pulmonary microvascular pressure noted. Increased surface tension and lung wet:dry ratio were observed.	We conclude that whereas the Veh in which Det is dissolved causes no significant permeability change, Veh plus Det in combination with an elevated Ppa produces a significant change in lung microvascular permeability, the extent of which is somewhere between baseline and the changes observed after alloxan. These experiments suggest that the combination of reducing perivascular hydrostatic pressure and increasing microvascular hydrostatic pressure in the standing unanesthetized sheep presents conditions favorable for

							an increase in microvascular permeability.
Warisnoicharoen et al., 2003. Toxicological evaluation of mixtures of nonionic surfactants, alone and in combination with oil. **Identified in Initial Literature Search**	Polyoxyethylene-10-oleyl ether, polyoxyethylene-10- dodecyl ether, N,N-dimethyl-dodecylamine-N- oxide: nonionic detergents	*In vitro*, 16HBE14o- cells, human bronchial cell line	Media, 0.001,0.01, 0.05, 0.1, 0.25, 0.5, 1.0, and 10.0 mg/mL	Cells were exposed to 0.1 mL of microemulsion or micellar solution, or 0.1mL of PBS for 30 minutes. Cells were then rinsed and incubated for 60 minutes with MTT solution in MEM (without phenol red). Surface tension was measured by the Wilhelmy plate technique.	N/A	On a molar basis, C12AO was the least toxic, followed by C18:1E10 and C12E10, which had similar IC50s. Microemulsions prepared with both the C12 surfactants produced the largest area of microemulsion existence when solubilizing the smaller molecular volume oils. All C12E10- and C12AO-containing systems were toxic at concentrations around or below their critical aggregation concentrations (as determined by surface tension measurements).	It is proposed, therefore, that the reduction in toxicity seen with the systems prepared with C18:1E10 and containing soybean oil, Miglyol 812, or ethyl oleate is a consequence of the diminished capacity of the surfactant aggregates to incorporate into the surfactant monolayer of the microemulsion amphiphilic components of the cell membrane, such as phospholipid.

iii. Studies in humans

In general, the database captured by both the Initial Literature Search and the Supplemental Literature Search, consists of older studies. These studies also differ in quality (*i.e.*, study design, technologies, and or reporting).

Epidemiological studies, associated with acute respiratory toxicity, either were not identified or did not meet any of the PECO criteria outlined in the Initial Literature Search or the Supplemental Literature Search. Both of these searches identified one older human volunteer study described by Obernour et al. (1963), in which a significant decrease in pulmonary compliance occurred with exposure the detergent Defomaire ([REF _Ref46548446 \h * MERGEFORMAT]).

Table [SEQ Table * ARABIC]. Population: Human studies on general surfactants.[a]

Reference	Product/Agent	Exposure/Comparator	Clinical Outcomes/Toxicities
Obernour et al., 1963	Defomaire	Normal healthy volunteers administered 3 mL siliconized respiratory detergent via nebulizer for 6 minutes / baseline (aerosol droplet size was not noted)	Pulmonary compliance was measured and resistance calculated. There was a significant decrease in pulmonary compliance with increased tissue resistance with exposure to aerosolized Defomaire.

[a] Bold font represents reference identified in the Initial Literature Search.

iv. Studies in animal, in vitro, and ex vivo models

Decreased pulmonary compliance is the result of an increase in surface tension in the alveoli that occurs with inhaled detergents. *In vivo* animal and *in vitro/ex vivo* studies are summarized in [REF _Ref46548546 \h * MERGEFORMAT] and [REF _Ref46548653 \h * MERGEFORMAT], respectively, according to the PECO criteria that are used to highlight critical information and/or gaps in knowledge base ([REF _Ref46548287 \h * MERGEFORMAT]).

Many of the *in vivo* studies (12 of 15) identified in the Initial Literature Search and additional studies identified in the Supplemental Literature Search, evaluated the anionic detergent, dioctyl sodium sulfosuccinate (DOSS) (cited in [REF _Ref46548546 \h * MERGEFORMAT]). As identified in the Initial Literature Search, in all the animal species evaluated (*e.g.*, dogs, sheep, rabbits, rats), inhaled DOSS increases in surface tension was associated with increased membrane permeability. This effect was demonstrated in a number of the studies reported in [REF _Ref46548546 \h * MERGEFORMAT] by using radiolabeled diethylenetriamine pentaacetic acid (DTPA), a small hydrophilic molecule, which cannot readily permeate intact cell membranes, to evaluate alveolar cell permeability. In a study that evaluated lung histopathology following exposure (~4 hours) of dogs, damage to the alveolar cells or lung architecture was not observed (Nieman and Bredenberg, 1985). Selected studies showed a dose-dependent increase in surface tension in pulmonary surfactant extracted from dogs treated with a nonionic surfactant, as described by Modell et al. (1969). Although this study was conducted some time ago, Modell et al. (1969) also demonstrated that the response to the pulmonary surfactant following 8-hour inhalation exposure did not produce much of an effect.

The information on particle/aerosol droplet size was not always provided in the *in vivo* animal studies, and this parameter was not relevant in the *in vitro* systems used to evaluate pulmonary surfactant function. Also, in many of the studies that reported particle size, mass median aerodynamic diameter (MMAD) was <10 μm. Although there were a number of *in vitro* and *ex vivo* models that provided information for supporting a mode of action for the acute pulmonary toxicity *via* the substances' ability to damage the pulmonary surfactant and increase surface tension through changes in membrane permeability, only a few studies evaluated general surfactants in relevant cells lines (*e.g.*, lung cells). One *in vitro* study was identified in the Initial Literature Search in which a human bronchial cell line (16HBE14o) was utilized (Warisnoicharoen et al., 2003); however, in the Supplemental

Literature Search, there was a study that evaluated the toxicity of identified substances in human lung carcinoma cell line (A549) (Tsujino et al. 1990). The information provided in these studies supports integrating an *in vitro* assay for screening the lung toxicity of general surfactants using a lung specific model system.

Table [SEQ Table * ARABIC]. Population: Animal studies on general surfactants.[a]

Reference	Product/Agency	Exposure /Comparator	Outcomes/Toxicities
Damon et al., 1982	Polyethylene glycol p-isooctylphenyl ether (Triton X-100)/^{3}H-Triton X- 100	Hamster, nose-only (NO) inhalation (nebulizer) aerosol of 10% Triton X-100 in ethanol, 0, 800, 1400, 1900, 2500, with 800–3100 µg estimated lung burden, and hamsters lavaged with 0.01, 0.05, 0.06, 0.075, 0.10% Triton X-100 solution in saline (lung burden = 800-3100 µg); Aerosol Mass median aerodynamic diameter (MMAD) = 1.47–1.51 µm, GSD=1.84–1.91, mass concentration of 3.0 mg/liter	Similarity in LD_{50} and lung burden between the two routes of exposure, with lung histopathology changes showing the nature and distribution differed between these two routes; with lesions of pulmonary edema following lavage administration.
Evander et al., 1988	Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, inhalation of aerosol, 5% solution DOSS for 5 min, followed by ^{99m}Tc-DPTA via aerosol.	P_{O2}, P_{CO2} and clearance of ^{99m}Tc-DPTA measured. Increased clearance of ^{99m}Tc- DPTA, with no effect on pressure or compliance. Change in clearance of ^{99m}Tc-DPTA is a sensitive indicator of altered surfactant function.
Evander et al., 1994	Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, inhalation of aerosol, 5% solution DOSS for 5 min, followed by ^{99m}Tc-DPTA via aerosol. DOSS concentrations: 0, 0.125, 0.25, 0.5, and 2%; with MMAD = 1.7µm, ^{99m}Tc-DTPA MMAD = 3.3µm	No effect on blood pressure, Pa_{O2}, Pa_{CO2}, or compliance. DOSS induces a biexponential clearance course of ^{99m}Tc-DTPA due to increased transfer across the alveolocapillary, which is dependent on the dose of DOSS. The effect of detergent was partly reversible.
Jefferies et al., 1988	Dioctyl sodium sulfosuccinate (DOSS)	Rabbits, inhalation of aerosol—20 mL 1.5% solution for 20 minutes followed by ^{99m}Tc-DTPA aerosol for 1–2 minutes with free breathing, conventional ventilation, or high- frequency oscillation ventilation.	Clinical signs of respiratory distress noted in all DOSS- exposed rabbits; acidosis and declining oxygenation increased with time; lung volume (pressure-volume curve) was decreased in rabbits exposed to DOSS compared to vehicle-treated. ^{99m}Tc-DTPA clearance increased significantly in exposed rabbits regardless of modes of ventilation.

		Aerosol contained particles with mass median aerodynamic diameter = 0.6μm and GSD = 1.97μm.	
John et al., 1997	Dioctyl sodium sulfosuccinate (DOSS)	Rabbits, aerosol inhalation of 2% DOSS via conventional or large tidal volume ventilation followed by a recovery period. ^{99m}Tc- human serum albumin (HAS) was administered to evaluate clearance mechanisms.	Lung mechanics and arterial blood gas determinations were evaluated. DOSS decreased the clearance half- life of HAS.
Martinez & Brown, 1991	Polyoxy- ethyleneamine (POEA) or polysorbate-80; non-ionic surfactants	Rats, administration directly into trachea; POAE (7%) at 0.1, 0.2, and 0.4 mL, and polysorbate-80 (7%) at 0.1 and 0.2 mL.	Administration of POEA (within 24 hr) produced 20, 70, 100% death at 0.1, 0.2, and 0.4 mL, respectively, with increased lung weight and damage (subjective scoring), while polysorbate- 80 did not. No explanation for the differences was noted.
Modell et al., 1969	Alevaire	Dogs, endotracheal catheter pass via a tracheostomy for measurements from one lung, and ultrasonic nebulizer with Alevaire or ethyl alcohol continuously for 8 hours.	There was a significant decrease in arterial oxygen tension with a slight decrease in $PaCO_2$ and corresponding increase in pH. Surface tension-surface area loop showed normal hysteresis in all cases, unlike reported in the *in vitro* study (see [REF _Ref46548653 \h * MERGEFORMAT]).
Nieman et al., 1985	Dioctyl sodium sulfosuccinate (DOSS)	Dogs, aerosol inhalation via ventilator, 15 mg/kg in 1% solution, total volume of 1.5 mL/kg administered over 30–45 min. The study measured arterial pressure (femoral, pulmonary), blood gasses, hemoglobin, and pH. Microscopic examination and edema assessment (pulmonary extravascular water volume [PEWV])	Partial diffuse lung collapse increased over time with progressive decrease in lung volume (end of expiration). Edema fluid (foam) in small airways following lung collapse; by 2 hr, extensive foam in major bronchi and distal trachea. Destabilization and large changes in size of subpleural alveoli were observed. Compared to controls, PEWV increased in animals killed 2 hours following aerosol administration. Decreased surface tension and surfactant activity measured by Wilhelmy balance—see [REF _Ref46548653 \h * MERGEFORMAT].

		measured by gravimetric technique. For the *ex vivo* study, lung samples were taken 30 and 120 minutes after aerosol inhalation (see [REF _Ref46548653 \h * MERGEFORMAT]) Mean = 3 µm (range, 0.5–15 µm)	
Nieman et al., 1990	Dioctyl sodium sulfosuccinate (DOSS)	Dog, aerosol inhalation via ventilator, 15 mg/kg in 1% solution; a total volume of 1.5 mL/kg was administered over 30–45 min. After delivery of DOSS, an exposure to ^{99m}Tc-DTPA (particle size <1 µM, diethylenetramine-pentaacetic acid) over 5 min. Mean = 3 µm (range, 0.5–15 µm)	Arterial O_2 tension decreased and peak airway pressure increased following treatment. ^{99m}Tc-DPTA clearance was significantly faster in exposed animals compared to controls. It is noted that the increase in solute flux suggests deactivation of the surfactant by plasma proteins originating from the bronchiolar epithelium; occurs in the early stage of adult respiratory distress syndrome (ARDS) and represents a plausible mechanism for the later alveolar flooding.
Nilsson et al., 1992	Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, aerosol inhalation via ventilator, 1% solution at 5 min, with monitoring for 30 min. Rabbits were exposed to aerosolized ^{99m}Tc-DTPA or ^{99m}Tc-albumin to monitor clearance. One group was co-administered oleic acid.	Clearance of ^{99m}Tc-DPTA, arterial pressure, Pa_{O2}, and Pa_{C02}, were evaluated. Tc- albumin clearance was slightly lower with DOSS, and much lower with DOSS + oleic acid. ^{99m}TC-DTPA clearance was significantly lower than control with either DOSS or DOSS + oleic acid. DOSS alone did not affect Pa_{O2}, Pa_{CO2} or compliance, but administration of oleic acid resulted in a reduction in Pa_{O2} and an increase in $PaCO_2$.

Nilsson et al., 1993	Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, aerosol inhalation via ventilator, 1% solution for 5 min. The DOSS aerosol was followed by an immediate intratracheal instillation of ^{99m}Tc-DTPA, ^{99m}Tc-sestamibi, or ^{99m}Tc-HIDA.	Surfactant dysfunction was induced by DOSS aerosol. Clearance of ^{99m}Tc-DPTA was substantially increased following DOSS, but only slightly with ^{99m}Tc-sestamibi, and no difference using ^{99m}Tc-HIDA. DOSS had no significant effect on Pa_{O2}, Pa_{CO2} in these groups. Damage to the surfactant seems is associated with the transfer of small water-soluble, but not high-lipid soluble molecules.
Nilsson et al., 1997	Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, inhalation, aerosol via ventilator, 2% solution for 5 minutes, resulting in deposition of approximately 10 µL of fluid. Instillation of natural surfactant to determine if damage from DOSS could be attenuated. MMAD = 1.7 µm	Tracheal instillation of natural surfactant attenuated the effect of DOSS on the pulmonary clearance of ^{99m}Tc-DTPA.
Rao & Das, 1994	Dioctyl sodium sulfosuccinate (DOSS)	Rat, whole-body aerosol inhalation, 100, 200, 300, 400, or 500 mg over 10 min to 1 hour.	Thirty min post-exposure, pulmonary edema was observed in 3/5 rats at the high dose only.
Taskar et al., 1995	Dioctyl sodium sulfosuccinate (DOSS)	Rabbits, aerosol inhalation exposure of 2%, followed ^{99m}Tc-DTPA. MMAD = 1.7µM	The clearance kinetics of ^{99m}Tc-DTPA following large tidal volume ventilation are qualitatively different with exposure to DOSS.

Wang, 1993	Dioctyl sodium sulfosuccinate (DOSS)	Sheep, aerosol inhalation of 15 mg/kg DOSS in 30 mL of vehicle (saline + ethanol) for 1hr followed by 12 hr of sample and 12 hr of recovery. Mean = 3 µm (range, 0.5–15µm)	No change in Pa_{O2}, Pa_{CO2}, pH, with a small effect on pulmonary microvascular pressure was noted. Increased surface tension and lung wet:dry ratio was observed.

[a] Bold represents reference identified in the Initial Literature Search.

Table [SEQ Table * ARABIC]. Population: *In vitro* or *ex vivo* studies on general surfactants.[a]

Reference	Product/Agent	Exposure/Comparator	Outcomes/Toxicities
Bachofen et al., 1979	Triton X-100	*Ex vivo*, isolated perfused rabbit lungs, alveolar lavage 0.01% Triton X- 100 solution / baseline levels or unexposed.	Total lung capacity (TLC): progressive collapse of alveoli, with most alveoli collapsed at 40% TLC. Pressure-volume curves: Exposed lungs had a shift in the deflation limb. Morphological evaluations: no gross effects on alveolar septa, some localized damage of squamous alveolar epithelium, focalized collapsed areas, with macrophages.
Ehrhart et al., 1981	Oleic acid	*Ex vivo:* Isolated lungs perfused at constant pressure with heparinized blood with exposure to various concentrations of oleic acid (0, 1, or 4 μL/kg) in the perfusate.	Weight gained increased linearly over 1–3 h, more rapid at the higher oleic acid dose. Total lobe weight gain, pulmonary vascular resistance, decrease in arterial O_2 partial pressure were greater in the 45- vs 1- μL/kg group.
Ekelung et al., 2004	Polyethyleneoxide (PEO) surfactants: $C_{12}E_8$, $C_{12}E_{(23)}$ (BRIG 35), $C_{14}E_8$, $C_{16}E_8$, $C_{16}E_{(20)}$ (BRIG58), $C_{18}E_{(20)}$ (BRIG 78), M-C18E(20) (MYRJ 49), M-$C_{18}E_{(40)}$ (MYRJ 52), M- $C_{18}E_{(100)}$ (MYRJ 59) (defined in Attachment C, Section 2)	*In vitro*: Tensiometry to measure surface tension, and effects on Caco-2 cells and in pig nasal mucosa by Ussing chamber experiments: Incubation with concentrations ranging from 10^{-5} to 10 mM, measurements of transepithelial electrical resistance (TEER), and transport.	Surface tension increased with increasing alkyl chain length, and surfactants showed decreases in TEER, with marked increases in mannitol permeability and trypan blue accumulation. Surfactants with long PEO head groups are less toxic than analogs with short PEO groups.
Fischer et al., 2012. Pilot study	Surface active substances (#1-12), not identified in this pilot study—fluorocarbon molecules with side chains with 4 carbons (#1, 8), 6 carbons (#7, 9, 11), 8 carbons (#2-6, 10, 12).	Rat isolated perfused lung model (IPLM) exposed to 45–3125 μg/lung / n- hexane, compared to *in vivo* acute inhalation toxicity data (OECD TG 403) at 20-mg/L limit concentration) (studies not described in this or cited)	IPLM parameters included respiratory, atelectasis, and measure of reversibility. The acute inhalation toxicity test induced breathing pattern and pathology. Note: The changes to respiratory function and lung pathology that occurred *in vivo* correlated with changes in the IPRL.
Hall et al., 1992	Oleic acid	*In vitro*: Surfactometry used to measure surface tension *Ex vivo*: perfused rat lungs: instillation with 4, 10, or 20 mg oleic acid dispersed by sonication in 2 mL of saline / solvent controls.	*In vitro*: oleic acid inhibited pulmonary surfactant activity and increased surface tension. *Ex vivo*: Instillation of oleic acid resulted in altered deflation pressure-volume characteristics, suggesting an effect on pulmonary surfactant.

Meinert et al., 1992	9 Different perfluoralkylated surfactants-with same fluorophilic tail and hydrophilic heads but different prolongators.	*In vitro*: interfacial-tensiometer Lecomte duNouy method using a rigid platinum ring. Toxicity evaluated in HeLa cells (epithelic cells from cervix) and Molt 4 cells (T-cell leukemia cell line). Surfactants dissolved in isotonic buffer (10% w/v) were identified as % (w/v) in culture (0.04 to 2.5).	In the cell culture, surfactants caused a significant reduction in proliferation, depending on the concentration and chemical nature of the agent. One surfactant caused a >50% inhibition produced by concentrations greater than 0.16% in both cell lines. No direct correlation of biocompatibility with surface tension or interfacial tension was noted.
Modell et al., 1969	Alevaire	*In vitro*: Wilhelmy balance to measure surface tension using pulmonary surfactant extracted from dogs. Normal saline vs. Alevaire (1 to 30 mL added to 150 mL of saline) / ethyl alcohol (1 to 100 mL in 150 mL of saline).	No concentration-related differences in surface tension—surface area loop, with progressive decrease in surface compressibility of the film (i.e., narrowing hysteresis loop) that were then reversed. Surface tension-surface area loop showed greater response compared to *in vivo* study (see [REF _Ref46548546 \h * MERGEFORMAT]).
Nieman et al., 1985	Dioctyl sodium sulfosuccinate (DOSS)	*Ex vivo*, minced dog lung extracts, taken 30 and 120 minutes after aerosol inhalation (see *in vivo* study in Table 20). Mean = 3 μm (range, 0.5–15 μm).	Diminished surfactant activity measured by Wilhelmy balance. Used with *in vivo* study in [REF _Ref46548546 \h * MERGEFORMAT] to provide evidence that pulmonary edema can be induced by increased surfactant surface tension.
Sørli et al., 2015	1% POTS (hydrolysates and condensates of 1H,1H, 2H, 2H-perfluorooctyl-trialkoxysilane in 2-propanol; product equivalent to non-absorbing floor materials-nine spray products containing perfluoracrylate, alkylsilan/siloxane, perflurosilan/ siloxane.	Capillary surfactometer, Alveofact (4 mg/mL) was incubated with the products diluted in original solvents or solvent alone. Dose of POTS (by volume) was added to mixtures, with solvents evaporated. The sample preparations were evaluated in a concentration-dependent manner, but only by dilution, so actual concentrations are not provided.	All products that were toxic in mice exposed via inhalation and inhibited the pulmonary surfactant function *in vitro*. Two products that were negative *in vivo* were negative *in vitro*. Two of three false positives were at the highest concentration. Negative predictive value was 100%; positive predictive value was 57%.
Tsujino et al., 1990	Tween 80	*in vitro*, growth inhibition (A549, H69, PC14, PC14/CDDP, and KB cell lines) A549 (human lung carcinoma), H69 (human small-cell carcinoma), plus PC14,	Inhibited cell growth, increased uptake and accumulation of etoposide, but no change in uptake of hydrophilic compound danorubicin was observed. The disruption of cell membrane by a

		PC14/CDDP and KB cell lines. Cells were treated with etoposide, Tween 80, or etoposide + Tween 80. Survival was measured after 5 days.	detergent would allow lipotropic drugs to enter.
Warisnoicharoen et al., 2003	Polyoxyethylene- 10-oleyl ether, polyoxyethylene- 10-dodecyl ether, N,N-dimethyl- dodecylamine-N- oxide: nonionic detergents	*In vitro*, 16HBE14o- human bronchial cell line; media, 0.001,0.01, 0.05, 0.1, 0.25, 0.5, 1.0, and 10.0 mg/mL.	On a molar basis, C12AO was the least toxic, followed by C18:1E10 and C12E10, which had similar IC_{50}s. Microemulsions prepared with both the C12 surfactants produced the largest area of microemulsion existence when solubilizing the smaller molecular volume oils. All C12E10- and C12AO-containing systems were toxic at concentrations around or below their critical aggregation concentrations (as determined by surface tension measurements).

[a] Bold represents reference identified in the Initial Literature Search.